EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON THE REPRODUCTIVE PHYSIOLOGY OF *ANASTREPHA SUSPENSA* (LOEW) (DIPTERA: TEPHRITIDAE)

By

LIKUI YANG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2000

ACKNOWLEDGMENTS

I would first like to thank my major professor, Dr. Herbert N. Nigg, for his criticism, guidance, and encouragement. I would also like to thank the members of my supervisory committee-- Drs. Larry W. Duncan and Fred G. Gmitter, especially Drs. James L. Nation and Simon S. Yu -- for their helpful advice and encouragement. Grateful acknowledgment is given to Drs. Armen C. Tarjan and Grover C. Smart, Jr. Without their assistance and consultation, I would not have continued at the University of Florida. I also thank Dr. Alfred M. Handler, USDA, Gainesville, for his helpful suggestion and kindly gifts, antibody and cDNA probe. I extend thanks to the following people who, in one way or another, contributed to the completion of this work: Sam E. Simpson, Dr. Louis E. Ramos, Nadine W. Green, Jeannette I. Barnes, Carmon G. Green, and Fahiem E. Elborai for their friendship and assistance in the laboratory; Diann C. Achor for teaching me to use SEM; Dr. Shailaja Shivprasad and Cecile J. Robertson, who helped me to run Western and Northern blotting and let me use equipment in their laboratory; Barbara Thompson for editing the dissertation; Terri Appleboom and Dr. Monica Lewandowski for preparing slides; Pamela K. Russ and Jamie L. Chastain for library service. Finally, I would like to extend my special gratitude and thanks to my wife, Lingxia Zhao, and our lovely son, Kevin Yang. Without their love, understanding, and support, this would not have been possible.

TABLE OF CONTENTS

page

ACKNOWLEDGMENTS ii

LIST OF TABLES v

LIST OF FIGURES viii

ABSTRACT xi

CHAPTERS

1 INTRODUCTION 1

2 REVIEW OF LITERATURE 5

Vitellogenin and Egg Development 5
Diet and Egg Development 14
Male Insects and Egg Development 17
Effects of Chemicals on Egg Development 18
Proteolytic Enzymes in Insects 24
Insect Esterases Related to Reproduction 33

3 EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON THE SURVIVAL AND REPRODUCTION OF *ANASTREPHA SUSPENSA* 42

Introduction 42
Materials and Methods 44
Results and Discussion 50

4 MORPHOLOGICAL EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON OVARIAN DEVELOPMENT OF *ANASTREPHA SUSPENSA* 89

Introduction 89
Materials and Methods 91
Results and Discussion 94

5 EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON PROTEINASE ACTIVITIES OF FEMALE *ANASTREPHA SUSPENSA* 108

Introduction 108
Materials and Methods 110
Results and Discussion 115

6 EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON GENERAL ESTERASE ACTIVITIES IN *ANASTREPHA SUSPENSA* 134

Introduction 134
Materials and Methods 135
Results and Discussion 137

7 EFFECT OF SODIUM TETRABORATE AND IMIDACLOPRID ON YOLK PROTEIN SYNTHESIS IN *ANASTREPHA SUSPENSA* 146

Introduction 146
Materials and Methods 147
Results and Discussion 153

8 SUMMARY AND CONCLUSION 164

REFERENCES 168

BIOGRAPHICAL SKETCH 197

LIST OF TABLES

Table page

2-1. Summary of vitellogenin/or vitelline in insects 37

2-2. Protein requirement and egg development in female adult insects 38

2-3. The types of proteinases determined in insects 39

3-1. LC_{50} s of sodium tetraborate and imidacloprid to *A. suspensa* treated as newly emerged adult by feeding 24 hr (48 hr mortality) 61

3-2. Mean mortality of *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24 hr. 62

3-3. Mean mortality of *A. suspensa* treated as newly emerged adult with imidacloprid by feeding 24 hr. 63

3-4. LT_{50} s of sodium tetraborate to *A. suspensa* treated as newly emerged adult by feeding 24 hr. 64

3-5. LT_{50} s of imidacloprid to *A. suspensa* treated as newly emerged adult by feeding 24 hr. 65

3-6. Mean fecundity and fertility of *A. suspensa* treated as newly emerged adult with various concentrations of sodium tetraborate by feeding different periods. 66

3-7. Mean fecundity and fertility of *A. suspensa* treated as different age adult with 0.5% sodium tetraborate by feeding 24 hr. 67

3-8. Mean fecundity and fertility of *A. suspensa* treated as newly emerged adult with various concentrations of imidacloprid by feeding different periods. 68

3-9. Mean fecundity and fertility of *A. suspensa* treated as different age adult with 1.0 mg/l imidacloprid by feeding 24 hr. 69

3-10. Mean fecundity of *A. suspensa* treated as newly emerged adult with various concentrations of sodium tetraborate by feeding different periods. 70

3-11. Mean fecundity of *A. suspensa* treated as 10-d-old adult with various concentrations of sodium tetraborate by feeding different periods. 71

3-12. Mean fertility of *A. suspensa* treated with various concentrations of sodium tetraborate by feeding different periods. 72

3-13. LT_{50} s of sodium tetraborate to *A. suspensa* treated as newly emerged adult by feeding one week on choice or no choice food. 73

3-14. LT_{50} s of sodium tetraborate to *A. suspensa* treated as 10-d-old adult by feeding one week on choice or no choice food. 74

3-15. Mean fecundity of *A. suspensa* treated as newly emerged adult by feeding one week with various concentrations of sodium tetraborate. 75

3-16. Mean fecundity of *A. suspensa* treated as 10-d-old adult by feeding one week with various concentrations of sodium tetraborate. 76

3-17. Mean egg hatch of *A. suspensa* treated as newly emerged adult for one week with various concentrations of sodium tetraborate. 77

3-18. Mean egg hatch of *A. suspensa* treated as 10-d-old adult for one week with various concentrations of sodium tetraborate. 78

4-1. Time course of ovary development of *A. suspensa* 97

4-2. Ovary dimensions of 7-d-old *A. suspensa* treated as newly emerged adult with different concentrations of sodium tetraborate by feeding 24 hr. 98

4-3. Ovary dimensions of 7-d-old *A. suspensa* treated at different ages with 0.5% of sodium tetraborate by feeding 24 hr. 99

4-4. Ovary dimensions of 7-d-old *A. suspensa* treated as newly emerged adult with different concentrations of imidacloprid by feeding 24 hr. 100

4-5. Ovary dimensions of 7-d-old *A. suspensa* treated at different ages with 1.0 mg/l imidacloprid by feeding 24 hr. 101

5-1. Proteinase activities in the midgut of 6-d-old female *A. suspensa*. 123

5-2. Proteinase activities in the ovary of 7-d-old female *A. suspensa.* 124

5-3. *In vitro* inhibition of midgut proteinase activities of 4-d-old female *A. suspensa.* 125

5-4. *In vitro* pesticide effects on proteinase activities in the midgut of 4-d-old female *A. suspensa.* 125

5-5. *In vitro* effect of potential proteinase inhibiting or activating compounds on the hydrolysis of BApNA, BTpNA, and LPNA by ovary homogenate from 7-d-old female *A. suspensa.* 126

6-1. General esterase inhibition by selected compounds in the abdomen and the whole body of both male and female *A. suspensa.* 142

LIST OF FIGURES

Figure page

2-1. Chemical structure of proteinase substrates used in this study 41

3-1. Oviposition pattern of *A. suspensa* 79

3-2. Mortality of male *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24 hr (A), 48 hr (B), and 168 hr (C) 80

3-3. Mortality of female *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24 hr (A), 48 hr (B), and 168 hr (C) 81

3-4. Mortality of male *A. suspensa* treated as 10-d-old adult with sodium tetraborate by feeding 24 hr (A), 48 hr (B), and 168 hr (C) 82

3-5. Mortality of female *A. suspensa* treated as 10-d-old adult with sodium tetraborate by feeding 24 hr (A), 48 hr (B), and 168 hr (C) 83

3-6. Mean fecundity of *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24 hr (A), 48 hr (B), and 168 hr (C) 84

3-7. Mean fecundity of *A. suspensa* treated as 10-d-old adult with sodium tetraborate by feeding 24 hr (A), 48 hr (B), and 168 hr (C) 85

3-8. Mortality of *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 7 d on choice food (A&C) or no choice food (B&D) 86

3-9. Mortality of *A. suspensa* treated as 10-d-old adult with sodium tetraborate by feeding 7 d on choice food (A&C) or no choice food (B&D) 87

3-10. Mean fecundity of *A. suspensa* treated as newly emerged adult (A&B) or 10-d-old adult (C&D) with sodium tetraborate by feeding 7 d on choice food or no choice food 88

4-1. Time course of ovarian development of *A. suspensa* 102

4-2. Ovarian development of 7-d-old *A. suspensa* treated as newly emerged adult with various concentrations of sodium tetraborate by feeding 24 hr 103

4-3. Ovarian development of 7-d-old *A. suspensa* treated at different ages with 0.5% sodium tetraborate by feeding 24 hr 104

4-4. Ovarian development of 7-d-old *A. suspensa* treated as newly emerged adult with various concentrations of imidacloprid by feeding 24 hr 105

4-5. Ovarian development of 7-d-old *A. suspensa* treated at different ages with 1.0 mg/l imidacloprid by feeding 24 hr 106

4-6. Ovary ultrastructure of 7-d-old *A. suspensa* treated as newly emerged adult with sodium tetraborate or with imidacloprid by feeding 24 hr 107

5-1. Time course of midgut proteinase activities of female *A. suspensa* 127

5-2. Time course of ovary proteinase activities of female *A. suspensa* 128

5-3. pH and proteinase activities in the midgut of female *A. suspensa* 129

5-4. pH and proteinase activities in the ovary of female *A. suspensa* 130

5-5. Midgut proteinase activities of female *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24 hr 131

5-6. Midgut proteinase activities of female *A. suspensa* treated as newly emerged adult with imidacloprid by feeding 24 hr 132

5-7. Ovarian proteinase activities of female *A. suspensa* treated as newly emerged adult with sodium tetraborate or imidacloprid by feeding 24 hr 133

6-1. Time course of general esterase activities in the abdomen (A) and whole body of male and female *A. suspensa* 143

6-2. General esterase activities in the abdomen (A&B) and whole body (C&D) of *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24 hr 144

6-3. General esterase activities in the abdomen (A&B) and whole body (C&D) of *A. suspensa* treated as newly emerged adult with imidacloprid by feeding 24 hr 145

7-1. Time course of yolk protein accumulation in the ovary of female *A. suspensa* . . 158

7-2. Immunoblot of yolk protein from different tissues of *A. suspensa* 159

7-3. Northern blot RNA hybridization of *A. suspensa* adult male and female specific tissues 160

7-4. Effect of sodium tetraborate on yolk protein synthesis in the ovary of female *A. suspensa* treated with different concentrations of sodium tetraborate (A) or treated different sex with 0.5% sodium tetraborate at different ages (B) 161

7-5. Effect of imidacloprid on yolk protein synthesis in the ovary of female *A. suspensa* treated with different concentrations of imidacloprid (A) or treated different sex with 1.0 mg/l imidacloprid at different ages (B) 162

7-6. Effect of sodium tetraborate and imidacloprid on yolk protein gene transcription in the ovary of female *A. suspensa* treated as newly emerged adults by feeding for 24 hr 163

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON THE REPRODUCTIVE PHYSIOLOGY OF *ANASTREPHA SUSPENSA* (LOEW) (DIPTERA: TEPHRITIDAE)

By

Likui Yang

May 2000

Chairman: Herbert N. Nigg
Major Department: Entomology and Nematology

Since bait spray with malathion to control *Anastrepha suspensa*, an economically important insect pest in Florida, has resulted in environmental and public protests due to environmental and human health concerns, interest has been shifted to compounds with environmental and toxicological safety, including boron compounds. The effects of sodium tetraborate and imidacloprid on survival and reproduction of *A. suspensa* were evaluated in the laboratory; the possible action mechanism of sodium tetraborate on egg development of *A. suspensa* was investigated.

The fecundity and fertility of *A. suspensa* were significantly reduced when flies were treated with 0.5% sodium tetraborate by feeding for 24 hr or 48 hr regardless of fly age. If treatment was continued over 168 hr, the effective concentration was reduced to

0.1%; higher concentrations killed most flies and terminated oviposition of survivors for 20 d after treatment. Sodium tetraborate (≤ 1.0%) was not repellent to *A. suspensa.*

Proteinase activities in the midgut of female *A. suspensa* were reduced for 4-5 d after treatment with 0.5% sodium tetraborate by feeding for 24 hr. Proteinase activities in the ovary of 7-d-old *A. suspensa* treated as a newly emerged adult were significantly reduced by 0.5% sodium tetraborate. General esterase activity levels were also affected in the abdomen of *A. suspensa* after treatment with 0.5% sodium tetraborate; esterase activities were increased in the female abdomen and decreased in the male abdomen.

Morphological examination showed that ovarian development of *A. suspensa* was inhibited when flies were treated with 0.5% sodium tetraborate for 24 hr and the inhibition resulted from reduction of yolk protein accumulation in oocytes. Vitellogenin gene transcription in the ovary of treated flies was delayed, and vitellogenin synthesis was subsequently reduced.

CHAPTER 1
INTRODUCTION

Anastrepha suspensa (Loew), commonly called the Caribbean fruit fly, the Caribfly and the guava fly, is one of several species of fruit flies which are indigenous to the West Indies (Weems, Jr., 1966). Caribbean fruit fly was first trapped and reported in Key West, Florida in 1931 but was believed to have been established in that area for many years before its discovery in 1965 (Nigg et al., 1994). Within a few years it spread throughout Florida and now infests more than 80 species of tropical and subtropical fruit in 23 plant families (Swanson & Baranowski, 1972; Von Windeguth et al., 1972). In an effort to detect, exclude, and control/eradicate this fruit fly, many basic studies related to biological, ecological, and biotechnical aspects of *A. suspensa* have been conducted (Nation, 1972, 1990, 1991; Mazomenos et al., 1977; Szentesi et al., 1979; Burk, 1983; Webb et al., 1984; Calkins et al., 1988; Sivinski & Heath, 1988; Greany & Riherd, 1993; Nigg et al., 1994; Nation et al., 1995; Handler, 1997).

Caribbean fruit fly is an economically important insect. The common guava, *Psidium guajava* L. (fam. Myrtaceae), is generally considered to be its most important host, but it also occasionally attacks commercial citrus, the major tropical and subtropical fruit in Florida (Burditt & Von Windeguth, 1975). Important economic losses result from quarantine restrictions imposed on Florida by important domestic and foreign export markets (Greany & Riherd, 1993). The Florida grapefruit industry has spent many millions of dollars on quarantine measures for *A. suspensa* (Nigg et al., 1994). These measures include fumigation of export fruit to Japan, malathion/bait ground applications, and trapping to support establishment of "fly free" zones (Simpson, 1993).

Alternate methods to certify commodities fly-free, including not only postharvest measures but also pre-harvest control strategies, have been developed (Greany & Riherd, 1993). Four postharvest treatment methods, methyl bromide fumigation (Benschoter, 1979, 1988), irradiation treatment (Von Windeguth, 1982), cold treatment (Benschoter, 1984; Sharp, 1993), and hot water treatment (Gould, 1988; Sharp & Hallman, 1992) have been applied to kill eggs and larvae of *A. suspensa* in fruit. Several pre-harvest control strategies have also been used to control this fruit fly. These methods include trapping (Lopez et al., 1971; Burditt, 1982; Heath et al., 1993), biological control (parasite release) (Baranowski & Swanson, 1971; Baranowski et al., 1993), sterile insect technique (sterile male release) (Burditt et al., 1974; Holler & Harris, 1991), and pesticides (Selheime & Sutton, 1969; Calkins, 1993; Simpson, 1993).

Since the larvae develop inside fruit (Martinez & Moreno, 1991), the emphasis in most control programs around the world has focused on adult pesticides, although alternative methods as described above are used to control adult fruit flies (Budia & Vinuela, 1996). A common method to control adult *A. suspensa* is a bait containing malathion (Selhime and Sutton, 1969; Calkins, 1993; Simpson, 1993). Because application of malathion over a wide area has resulted in environmental concerns and public protests, interest has shifted to compounds with low toxicity, including boron compounds. The discovery of a safe chemical that inhibits egg development at a low dose may lead to new control tactics for this fruit fly.

Vitellogenesis is a critical process during insect egg development, and involves vitellogenin synthesis, secretion, and uptake into the oocytes in most insects (Kunkel & Nordin, 1985; Hoffmann, 1995). Vitellogenin synthesis has been shown to be affected by many factors including juvenile hormone/ecdysteroids (Postlethwait & Handler, 1979; Huybrechts & De Loof, 1981; Cymborowski, 1992) and digested diet (Hagedorn, 1983; De Bianchi et al., 1985; Adams & Gerst, 1991). In addition, male insects may be another factor that affects egg development in some species of insects (Markow & Ankney, 1984; Bownes & Partridge, 1987).

The effect of chemicals, especially plant extracts, on the reproduction of insects has been investigated in various insect species (Rawlins et al., 1979; Friedel & McDonell, 1985; Chang et al., 1994; Lowery & Isman, 1996; Dong et al., 1997; Di Ilio et al., 1999). These chemicals interfered with vitellogenin synthesis, uptake (Song et al., 1990), or affected the titres of juvenile hormone (JH) and ecdysteroids which play an important role in the regulation of vitellogenin synthesis (Chang et al., 1994; Lowery & Isman, 1996), resulting in inhibition of egg development and reduction of egg production.

Sodium tetraborate and other boron compounds have been shown to control insect species including cockroaches (Bare, 1945; Cochran, 1995), flies (Lang & Treece, 1972; Hogsette & Koehler, 1994), termites (Grace, 1992), and ants (Klotz et al., 1997) by acting as a larvicide, adulticide, or sterilant. It was reported that when newly emerged screw-worm flies, *Cochliomgia hominivorax* (Coquerel), and house flies, *Musca* *domestica* (Linnaeus), were treated with 1% and 2.5-5.% sodium tetraborate in the diet, respectively, both fecundity and fertility were inhibited (Borkovec et al., 1969; Settepani et al., 1969). Nigg & Simpson (1997) showed that oviposition of *A. suspensa* was delayed up to 7 d after one feeding of sodium tetraborate. Imidacloprid is a new chloronicotinyl insecticide and has been shown effective against many economically important insect pests such as whiteflies, aphids, and beetles (Palumbo et al., 1996; Boiteau et al., 1997).

The purpose of this study was to collect data on the effects of sodium tetraborate and imidacloprid on survival, egg production, and egg hatch of *A. suspensa*, as well as on vitellogenin synthesis and activities of proteinases and esterases in *A. suspensa*. Additionally, the morphological changes in the ovarian development induced by these two chemicals were examined. These data may help the design of pesticides with a more subtle mode of action and a lower dose regimen than currently used methods.

CHAPTER 2
REVIEW OF LITERATURE

Vitellogenin and Egg Development

General Characters of Vitellogenin

Vitellogenesis is the most important metabolic event in the reproduction of insects and is regulated by a complex series of hormonal interactions. Vitellogenesis involves the production of vitellogenin, yolk protein precursors, and their entry into the oocyte (Hoffmann, 1995). Vitellogenin was first detected only in the female insects (Telfer, 1965; Pan et al., 1969). Consequently, it was recognized as a female specific protein which is sequestered by vitellogenic oocytes in the ovaries and is organized into protein yolk bodies. Yolk proteins serve as an important nutrition source for embryogenesis. In view of their function, the term "vitellogenin" was adopted for such proteins (Pan et al., 1969). Yolk protein modified from vitellogenin is termed vitellin and has also been loosely called vitellogenins (Chen et al., 1978).

Vitellogenins are characterized as oligomeric glycolipophosphoproteins consisting of one or more subunits. Besides structural similarities, these proteins have been found to be immunologically related in many species. In some species, endogenous proteolytic cleavage changes the pattern of vitellin peptides compared to vitellogenin.

Also, their lipid and carbohydrate moieties may have subtle differences through the processing of phosphorylation and glycosylation (Kunkel & Nordin, 1985; Rina & Mintzas, 1987).

Because these proteins are concentrated in the egg and are relatively easy to be purified, they are excellent material for the study of the regulation of their synthesis from the molecular, developmental, and physiological points of view (Hagedorn & Kunkel, 1979).

Synthesis, Secretion, and Uptake of Vitellogenin

Vitellogenin synthesis is affected by many factors. The involvement of juvenile hormones (JHs) and ecdysteroids in the process of egg maturation was first reported by Wigglesworth (1936) in *Rhodnius prolixus* (Stal) and Hagedorn & Kunkel (1979) in *Aedes aegypti* (Linnaeus), respectively. Since then, many studies have been conducted, and the results showed that both JHs and ecdysteroids could stimulate vitellogenin synthesis during egg development (Thomas & Nation, 1966; Bell, 1969; Postlethwait & Handler, 1979; Bowne et al., 1987; Cymborowski, 1992; Hoffmann, 1995). Further studies indicated that JHs stimulated normal synthesis and uptake into the ovary, but abnormal synthesis by the fat body; while ecdysteroids had no effect on the ovary but induced normal synthesis of vitellogenin by the fat body (Hardie, 1995). In mosquito, *A. aegypti*, egg development neurosecretory hormone (EDNH), corpus cardiacum stimulating factor (CCSF), and oostatic hormone were also involved in vitellogenin synthesis (Lea, 1967; Lea & Van Handel, 1982; Borovsky, 1988).

For most insects, vitellogenin synthesis is restricted to the fat body of the adult female insect. After synthesis, vitellogenin is released into the hemolymph for transport to the maturing oocytes (Telfer, 1965; Engelmann, 1969a; Hagedorn & Judson, 1972; Kunkel & Nordin, 1985). Further studies showed that in some insect species, especially Diptera, vitellogenin can also be synthesized in the ovaries (Ono et al., 1975; Fourney et al., 1982; Zhai et al., 1984; Rina & Mintzas, 1988). Within the ovaries, the follicular epithelium is the site of vitellogenin synthesis (Chia & Morrison, 1972; Brennan et al., 1982); and, in *Stomoxys calcitrans* (Linnaeus) and *A. suspensa*, yolk proteins appear to be exclusively synthesized by the developing ovaries (Houseman & Morrison, 1986; Chen et al., 1987; Handler, 1997). Usually, vitellogenin synthesis by the fat body is universally female limited; nevertheless, vitellogenins have also been found in various male insects at a low level compared to females (Huybrechts & De Loof, 1977; Lamy, 1984).

The mechanism of the export of yolk proteins from the fat body and the follicle cells involves the usual route through the Golgi apparatus and exocytosis at the plasma membrane (Kunkel & Nordin, 1985; Raikhel & Dhadialla, 1992). After being synthesized and leaving the ribosome, vitellogenin starts a long journey that ends in proteolysis and consumption by the developing embryo. During this journey, the protein moiety is modified in a number of ways, including carbohydrate and phosphate attachment as well as possible temporal changes in these moieties. The carbohydrate moieties may confer a certain degree of stability to the protein subunits, ensuring proper assembly or preventing aggregation prior to secretion (Kunkel & Nordin, 1985).

The onset of vitellogenic uptake is characterized by the formation of gaps and space between the follicle cells. Studies on uptake of vitellogenin by follicles demonstrated that vitellogenin uptake was specific for the oocytes and selective for vitellogenin, and that the process was saturable and sensitive to pH, temperature, and divalent cation concentration (Raikhel & Dhadialla, 1992; Hoffmann, 1995). The uptake of vitellogenin by the oocyte is a calcium-dependent receptor-mediated response, and the receptor binding of vitellogenin is also enhanced by JH (Kindle et al., 1988; Kulakosky & Telfer, 1989; Wang & Davey, 1992).

Vitellogenin in Insects

Vitellogenin and vitellin have been extensively studied in various insects (Hagedorn & Kunkel, 1979; Harnish & White, 1982; Kunkel & Nordin, 1985; Raikhel & Dhadialla, 1992). In some species, such as *Locusta migatoria* (Gellissen et al., 1976; Chen et al., 1978; Chinzei et al., 1981), *A. aegypti* (Hagedorn & Judson, 1972; Borosvky & Whitney, 1987), *Lymantria dispar* (Linnaeus) (Hiremath & Eshita, 1992), and some fly species (Bownes & Hames, 1977; Mintza & Kambysellis, 1982; De Bianchi et al., 1985; Handler & Shirk, 1988), the biochemical aspects of their vitellogenin-vitellin systems have been investigated in detail (Table 2-1). The vitellogenin in its native state has a size of about 400-600 kDa and consists of large (140-190 kDa) and small (40–60 kDa) subunits (Kunkel & Nordin, 1985)

In the female African migratory locust, *L. migatoria*, vitellogenin synthesis and appearance of vitellogenin in the hemolymph occurred on day 7 after adult eclosion and in the presence of mature males (Chen et al., 1978). Vitellogenin and vitellin with molecular weights of 265 kDa and 550 kDa, respectively, were identified. Both contained at least 8 polypeptides with molecular weights ranging from 52 kDa to 140 kDa. The lipid moieties were 8.3% and 6.7% by weight in vitellogenin and vitellin, respectively (Chen et al., 1978; Chinzei et al., 1981).

As in other lepidopterans, vitellogenin in the gypsy moth (*L. dispar*) is synthesized in the female larva during the last stadium (Ballarino et al., 1991). This vitellogenin, molecular weight of ~487 kDa, has been purified from the hemolymph of *L. dispar*. It consisted of 3 subunits of 190, 165, and 36 kDa, which were identical to those of yolk protein, and a 165 kDa subunit which might have derived from 190 kDa (Hiremath & Eshita, 1992).

In the mosquito, *A. aegypti*, vitellogenin was produced only after a blood meal. The molecular weights of vitellogenin and vitellin were about 350 kDa and 330 kDa, respectively. Upon denaturation with sodium dodecyl sulfate (SDS), vitellogenin and vitellin dissociated into several subunits with molecular weights ranging from 29 kDa to 220 kDa. *A. aegypt*i vitellin is high in aspartic and glutamic acids, and low in histine, methionine, cysteine, and tryptophan (Hagedorn & Judson, 1972; Borovsky & Whitney, 1987).

Three major yolk proteins (YP-1, YP-2, and YP-3) have been isolated and characterized from *Drosophila melanogaster* (Meigen). Their molecular weights as determined by SDS-polyacrylamide gel electrophoresis (PAGE) were 47.5 kDa, 46 kDa, and 44.5 kDa, respectively. Their isoelectric points were 6.5, 6.65, and 6.9 for YP-1, YP-2, and YP-3, respectively (Warrent et al., 1979; Mintza & Kambysellis, 1982). *In vitro* experiments with radioactive precursors have shown that, unlike other insects, both the fat body and the ovary of *D. melanogaster* were able to synthesize these yolk proteins. Like *D. melanogaster*, the house fly, *M. domestica*, can also produce vitellogenin in both the fat body and the ovary. At the beginning of vitellogenesis, the fat body appears to be the main site of synthesis of vitellogenin, while at the end of vitellogenesis, the role is taken over by the ovary (De Bianchi et al., 1985a). Vitellogenin and vitellin in *M. domestica* are composed of at least 5 polypeptides with apparent molecular weights of 54, 52, 51, 48, and 46 kDa (De Bianchi et al., 1985a). Adams & Filipi (1983) reported 3 types of polypeptides with molecular weights of 48, 45, and 40 kDa as subunits in *M. domestica* vitellogenin and vitellin.

In Tephritid flies, vitellogenin and vitellin have been studied in 3 species, *Ceratitis capitata* (Wiedemann) (Rina & Mintzas, 1987, 1988), *Dacus oleae* (Gmelin) (Levedakou & Sekeris, 1987; Zongza and Dimitriadis, 1988), and *A. suspensa* (Handler & Shirk, 1986, 1988). In *C. capitata*, 2 vitellins have been isolated and characterized; both have one subunit with molecular weight of 49 kDa and 46 kDa, respectively. Antibody to these two vitellins reacted partially with egg extracts of 3 *Drosophila* species (Rina & Mintzas, 1987). Similarly, vitellin containing 2 subunits with molecular weight of 47 kDa and 49 kDa was also identified from mature eggs of *D. oleae* (Levedakou & Sekeris, 1987).

A single major yolk polypeptide having a molecular mass of approximately 48 kDa was isolated and identified from *A. suspensa* (Handler & Shirk, 1988). This molecular mass was comparable to the 46- to 49-kDa vitellin subunits from *C. capitata* and *D. oleae* as well as various Drosophilids (Srdic et al., 1978). Unlike most other insects, but similar to the stable fly, *S. calcitrans* (Chen et al., 1987), *A. suspensa* yolk protein synthesis is almost totally restricted to the ovary. In the more closely related tephritid fly, *C. capitata*, significant levels of yolk protein synthesis occur in both the fat body and the ovary. The antibody to the purified 48 kDa yolk protein from *A. suspensa* recognized all 3 YPs from *D. melanogaster*. These data supported the assumption that the YPs of most higher Dipterans share similar molecular mass and are different from those of other insects, and this similarity in size presumably represents a conservation of structure within the group (Huybrechts & De Loof, 1981; Handler & Shirk, 1988).

Immunoreaction of protein from the hemolymph of both adult female and male with antiserum responding to the 48 kDa YP in oocytes indicated that YP production is probably not strictly sex specifically regulated in *A. suspensa* (Handler & Shirk, 1988). However, Northern blot analysis showed that yolk protein gene expression is completely female-specific, limited to the ovary and without apparent regulation by 20-hydroxy ecdysone (or JH) (Handler, 1997).

Vitellogenin, Vitellin, or Yolk Protein

Hagedorn and Kunkel (1979) reviewed the synthesis, transport, and uptake of proteins found in insect eggs. According to their definition vitellogenins comprise 60-90% of the soluble egg yolk proteins, are produced in the fat body, are transported to and taken up by the developing oocyte, and may be specific to females. Vitellins are formed in the ovary by metabolizing vitellogenins into smaller proteins. Vitellins make up yolk proteins (Hagedorn and Kunkel, 1979). Raikhel and Dhadialla (1992) used the same terminology for most insects except for the higher Diptera. The higher Diptera yolk proteins are smaller in molecular weight ranging from 44 to 51 kDa. Izumi et al (1994) adapted the same terminology, vitellogenin for large proteins, and vitellin, yolk protein for smaller proteins which are metabolites of vitellogenin and are sequestered by the ovary. In higher Diptera (fruit, flesh and house flies) ovarian proteins have been referred to as yolk proteins (Izumi et al 1994).

An early study of *D. melanogaster* used the term yolk proteins to describe the three proteins synthesized by both fat body and ovary (Bownes and Hames, 1978). Isaac and Bownes (1982) referred to these same proteins as vitellogenin. In *Calliphora erythrocephala* smaller molecular weight proteins were referred to as vitellins and the major 210 kDa MW egg protein as vitellogenin (Fourney et al, 1982). Other authors have referred to proteins produced by the follicle cells in *D. malanogaster* as vitellogenin and vitellin (Brennan et al., 1982); others that *D. melanogaster* hemolymph proteins are vitellogenin, but egg proteins are yolk proteins (Mintzas and Kambysellis, 1982). In the Diptera genus *Sarcophaga*, *Calliphora*, *Phormia,* and *Lucilia*, vitellogenin and yolk protein were used interchangeably (Huybrechts and De Loof, 1982). In *M. domestica* L. the term vitellogenin was used for blood borne proteins and for proteins outside the egg but which were destined for uptake by the egg. Once in the egg the term vitellin was used (Adams and Filipi, 1983; Bianchi et al., 1985; Bianchit and Pereira, 1987). Vitellin was the term for major egg protein in *S. calcitrans* L.; no vitellogenin in the hemolymph was formed (Houseman and Morrison, 1986). For *Aedes aegypti,* vitellogenins were described as high molecular weight proteins which were processed into smaller molecular weight proteins by the egg and stored as vitellin (Borovsky and Whitney, 1987). For *Ceratitis capitata* the proteins stored in the egg were termed vitellogenins and egg yolk polypeptides (Rina and Savakis, 1991).

C. capitata belongs to the Tephritidae family as does *A. suspensa*. In the first study of *A. suspensa* egg proteins, the terms yolk proteins and yolk polypeptides were used (Handler and Shirk, 1986). Polypeptide, of course, is a fancy term for protein. In subsequent *A. suspensa* studies the term yolk protein was used for proteins synthesized and stored by the ovary (Handler, 1997; Handler and Shirk, 1988). For *C. capitata*, Rina and Mintzas (1987) referred to what appeared to be the same protein as vitellogenin when found in the hemolymph and vitellin when found in the ovary. In *D. oleae*, the major egg protein was termed vitellin (Levedakou and Sekeris, 1987).

The term vitellogenin has been used in most cases to refer to hemolymph proteins with a molecular weight >100 kDa and destined to be or to have its subunits selectively taken up by the developing egg. Vitellins, yolk proteins and yolk polypeptides refer to proteins usually with molecular weights <100 kDa and found in high concentration in the insect egg. For *A. suspensa*, the primary site of synthesis of yolk proteins appears to be the egg and only proteins of <100 kDa molecular weight are produced. To avoid any confusion in terms, *A. suspensa* egg proteins in this dissertation are referred to as yolk proteins.

Diet and Egg Development

Many cell structural components and all of the enzymes that regulate the biochemical transformation are proteins (Dadd, 1985). Food sources containing adequate quality and quantity proteins are required by insects for regular development and reproduction (Ferro & Zucoloto, 1990). Protein requirement or not for egg development in some insect species was listed in Table 2-2.

Nutritional requirements for egg development in anautogenous and autogenous insects have been extensively studied (Spielman, 1971; Lea, 1972; Agui et al., 1985; Adams & Nelso, 1990; Wheeler & Buck, 1996). The terms autogenous and anautogenous were first applied to the mosquito, *Culex pipiens* (Linnaeus), and referred to their ability to develop eggs without or with a blood meal, respectively. These terms have been expanded to apply to a protein requirement for egg development that was not necessarily derived from blood (Adams & Nelson, 1990).

In *M. domestica*, when one anautogenous strain was provided with only carbohydrate, ovarian development was stopped at stage 4, the early phase of yolk deposition (Sakurai, 1978). A similar result was also found in other strains of anautogenous *M. domestica* (Agui et al., 1985; Adams & Nelson, 1990). Adams & Nelson (1990) also reported that in the new anautogenous strain of houseflies, about 7% were partially autogenous. This result was in agreement with the statement that most of the population of anautogenous species contain some autogenous members (Spielman, 1971).

Bownes & Blair (1986) showed that *D. melanogaster* female flies fed sugar-water do not mature their oocytes. Similarly, flies starved from the time of eclosion showed no vitellogenin synthesis and very few vitellogenic oocytes were seen. Northern blot analysis showed that the levels of yolk protein gene transcription were reduced in starved flies (Bownes et al., 1988).

For autogenous insects, nourishment for egg production can be accumulated during the larval stage. The amino acids held in storage proteins are transferred to vitellogenin, enabling autogenous egg development (egg development without a protein meal) (Wheeler & Buck, 1996), such as in *Sarcophaga bullata* (Huybrechts & De Loof, 1981) and *M. domestica* (De Bianchi et al., 1985). In some species of fruit flies, such as *C. capitata,* eggs may be produced without protein in the diet, but egg production increases when protein is supplied (Slansky & Scriber, 1985; Ferro & Zucoloto, 1990).

In *A. suspensa*, eggs were not produced when flies were maintained on only sugar food, but survival was comparable to protein fed flies (Nigg et al., 1995). These data indicated that like *Anastrepha obligua* (Macquart) (Braga & Zucoloto, 1981), *A. suspensa* has an absolute protein requirement for egg development.

In insects, protein consumption, vitellogenin synthesis, and egg development are associated with each other. It was reported that the average concentration of vitellogenin in the hemolymph of house fly, *M. domestica* was 0.14 μg/μl if flies were fed on sugar, but the peak of vitellogenin concentration was 16.6 μg/μl for protein fed fly (Adams & Gerst, 1991). In *S. bullata*, the level of vitellogenin was about 1 μg/μl at age of 4-7 d old when flies were maintained on sugar food (Huybrechts & De Loof, 1981). However, in sugar fed *Phormia regina* (Meigen) (Yin et al., 1989) and *A. aegypti* (Hagedorn, 1983), no vitellogenin was detectable. Thus, there appears to be 2 different vitellogenin patterns in sugar-fed anautogenous dipterans. In one group, vitellogenin production was initiated and low levels were found in the hemolymph. In the second group, vitellogenin production was not initiated and no vitellogenin was detectable in hemolymph (Adams & Gerst, 1991).

Further studies showed that the failure of ovaries to develop when anautogenous flies are held on a carbohydrate diet can probably be attributed to the interference with the neuroendocrine pathways which regulate JH and/or ecdysteroid synthesis/release (Adams & Nelson, 1990). It was reported that in *A. aegypti*, *D. melanogaster*, and *M. domestica*, diet modulated the induction of vitellogenin production by 20-hydroxyecdysone; and, in *M. domestica and D. melanogaster*, diet also modulated the effects of JH analogue (Gemmill et al., 1986; Bownes et al., 1988; Adams & Gerst, 1992). In the blowfly, *Phormia terraenovae* (R.D.), when females were kept on sugar, there was no detectable ecdysteroid 48 hr after eclosion. Feeding these flies with protein resulted in an ecdysteroid peak of 0.36 nmol/ml hemolymph, 72 hr after the start of feeding (Wilps & Zoller, 1989). In *M. domestica*, maintained on sucrose, the ecdysteroid level was not increased after sucrose pulse-feeding; but, when they were pulse-fed protein, ecdysteroid levels tripled in 24 hr (Adams & Gerst, 1991). Similar results were also found in *A. aegypti* (Hanaoka & Hagedorn, 1980) and *D. melanogaster* (Schwartz et al., 1985).

Male Insects and Egg Development

The presence of mature male insects may be an important factor that affects egg development in female insects (Gwynne, 1984; Markow & Ankney, 1984; Bownes & Partridge, 1987). It has been reported in many insects that the secretory products of the male accessory gland entered the hemolymph of female following mating and stimulated egg maturation and oviposition (Leopold, 1976; Chen, 1984).

In *Aedes* mosquito, it was found that secretions of these glands initiate various physiological reactions in mated female including the stimulation of oviposition and increase of egg fertility (Leahy & Craig, 1965). Friedel & Gillott (1976) reported that, in *Melanoplus sanguinipes* (Fabricius), the male accessory products stimulated oviposition and served as a protein source for the developing oocytes. In *D. melanogaster*, virgin females deposited a few eggs per day; after mating egg deposition increased to 40-80 eggs per day, depending on the stock and rearing conditions (Herndon & Wolfner, 1995; Kubli, 1996).

In *Drosophila silvestris*, ovarian development in immature ('young' adult) females was accelerated in the presence of mature male flies. Detailed analysis showed that acceleration appeared to result from a sex-specific substance on the food that the immature female consumed (Craddock & Boake, 1992; Boake & Moore, 1996).

In *D. melanogaster* (Gilbert & Richmond, 1982) and the cockroach, *Supella longipalpa* (Fabricius) (Chon et al., 1990), the enhancement of oocyte development appeared to be due to copulation. Copulation in *D. melanogaster* includes transfer of a sex-pheromone, a 36-amino acid peptide that increased progeny production. The sex-peptide appears to regulate oogenesis mainly by action on the ovary, i.e., by controlling the progression of vitellogenic oocytes (Soller et al., 1997). This sex peptide also stimulates JH synthesis in the *D. melanogaster* corpus allatum *in vitro* (Moshitzky et al., 1996).

Sivinsky & Smittle (1987) found that female *A. suspensa* prefer to mate with large males, and they may use transferred molecules during copulation as a nutrition source. Similar results were reported by Bownes & Partridge (1987) for *D. melanogaster* and *D. pseudoobscura*.

Effects of Chemicals on Egg Development

To reduce pest insect populations, chemical control is still a major strategy. However, the indiscriminate use of some pesticides caused environmental contamination and adverse effects on non target organisms (Di Ilio et al., 1999). To reduce the negative effects of applied chemicals, many plant-derived pesticides and more specific pesticides with low toxicity to humans have been used to control insect pests.

Benzylphenols and benzyl-1, 3-benzodioxole derivatives (BBDs), obtained by the chemical modification of biologically active constituents of Panamanian hard wood, *Dalbergia retusa* (Hemsley), inhibited reproduction in several insect species (Rawlins et al., 1979; Nelson & Hoosseintehrani, 1982; Chang et al., 1988, 1991; Dong et al., 1997). Rawlins et al. (1979) reported that when 0- to 5-d-old screw-worm flies were treated orally with benzylphenols and benzyl-1, 3-benzodioxoles at concentrations of

0.01-1.0%, complete sterility of female flies was obtained. When these BBD compounds were used to treat *A. aegypti*, the hatchability of eggs from treated females mated with normal males or from normal females mated with treated males was reduced (Nelson & Hoosseintehrani, 1982). Similar results were also reported for Oriental fruit fly, *D. dorsalis* by Hsu et al. (1989, 1990) and Dong et al. (1997). The histological analysis showed that BBD derivatives interfered with assembly of microtubules during spermiogenesis in *Dacus dorsalis* (Hendel), leading to abnormality of sperm development (Hsu et al., 1990; Dong et al., 1997).

In the Mediterranean fruit fly, *C. capitata*, when young adult flies were treated with BBDs compound at a concentration of 0.57 mg/g, ovarian growth in females was delayed by 9 d, and subsequent egg production and egg hatch were significantly reduced. Song et al. (1990) showed that benzodioxole J2581 (5-ethoxy-6-(4-methoxyphenyl) methyl-1,3-benzodioxole) prevented egg maturation when female *D. melanogaster* were treated with this compound at emergence, and the sectioned ovaries from treated females were rudimentary. Ultrastructural analyses revealed that the follicle epithelium was not patent, thus preventing the entry of vitellogenin into the oocytes. They also showed that the effect of J2581 in inhibiting vitellogenesis in *D. melanogaster* was reversed by treatment with (7S)-methoprene. The results obtained in this study suggested that J2581 did not interfere with JH binding to its receptor or its binding proteins. Similar results were obtained with the Mediterranean fruit fly, *C. capitata*, by Chang et al. (1991, 1994). Chang et al. (1994) also found that BBD compounds affected biosynthesis and release of JH from the *corpora allata* of the female *C. capitata*.

Azadirachtin and its derivatives, extracted from neem (*Azadiracta indica*) seed, have been shown to negatively influence the reproduction of insects in Orthoptera, Homoptera, and Diptera (Rembold, 1989; Lowery & Isman, 1996; Di Ilio et al., 1999). Effects of azadirachtin on metamorphosis, longevity, and reproduction of *C. capitata*, *D. dorsalis,* and *Dacus cucurbitae* (Coquillett) have been investigated (Stark et al., 1990). The results showed that azadirachtin significantly inhibited adult emergence when these 3 fruit fly species were exposed as late third instars or pupae to treated sand at concentrations of 10 to 14 mg/l. Egg production in adult *D. dorsalis* that had survived larval-pupal treatments with 1.85 mg/l azadirachtin was greatly reduced, but neither egg hatch nor growth and development of F1 progeny was affected. Similarly, when adult *C. capitata* were fed with 0.03% azadirachtin for 24 hr, fecundity was significantly reduced and slight reduction of longevity also was observed (Di Ilio et al., 1999).

In *Schistocerca gregaria* (Forskal), adults treated with azadirachtin had reduced quality and quantity of proteins in the hemolymph. Staining analysis showed that the synthesis and release of neurosecretion from A-type median neurosecretory cells of the brain were delayed in the treated females; therefore, the ovarian development was inhibited (Subrahmanyam & Rao, 1986). Rembold (1989) showed that no oviposition was found, and ecdysteroid titer was reduced in the ovaries of *Migatoria migratorioides* when treated with isomeric azadirachtins. Similar results were found in the green peach aphid, *Myzus persicae* (Sulzer), and the lettuce aphid, *Nasonovia ribisnigri* (Mosley), treated orally with 1% neem seed oil for 3 d (Lowery & Isman, 1996).

The triazine compound cyromazine, an insect growth inhibitor, reduced egg production of female sheep blow fly, *Lucilia cuprina* (Wiedemann), when 1- to 2-d-old adults were fed with 20 mg/l of cyromazine in water (Friedel & McDonell, 1985). In Mexican fruit fly, *Anastrepha ludens* (Loew), when young adult females were fed with cyromazine at concentrations of 0.1-5%, egg production was significantly reduced; when only male flies were treated, no effect was found (Martinez & Moreno, 1991; Moreno et al., 1994). However, Kotze (1992) reported that when adult *L. cuprina* was fed with cyromazine in water at concentrations up to 100 mg/l, both fecundity and fertility were not affected, but larval development of F1 was completely inhibited at 100 mg/l. A similar result was found in *M. domestica* (Pochon & Casida, 1983). Budia & Vinuela (1996) reported that when female adult, *C. capita*, were fed with 10 to 5,000 mg/l of cyromazine in water, oviposition was affected only at high doses (500 to 5,000 mg/l) with continuous treatment from adult emergence; larval development was significantly inhibited by the dose levels 10 - 10,000 mg/l.

Diflubenzuron (1-(4-chlorophenyl)-3-(2, 6-diflurobenzoyl)-urea), a chitin synthesis inhibitor, has been reported to disrupt development in some Diptera, causing malformed puparia (Wright, 1981) and decreased egg viability (Spates & Wright, 1980). Chang (1979) reported that fertility of *M. domestica* was reduced in the first 10 d posttreatment when adults were treated with 10 μg of diflubenzuron per fly by injection. For boll weevil, *Anthonomus grandis* (Boheman), when treated with 0.05% diflubenzuron plus 10 Krads of irradiation, sterility of both sexes was noted (Haynes et al., 1981). Sarasua & Santiago-Alvarez (1983) showed that continuously feeding *C. capitata* from emergence with 0.75% of diflubenzuron gave a significant reduction in fecundity, which was directly related to an interference with endocuticular deposition. In *A. suspensa*, fecundity and survivorship of first generation individuals between egg hatch and pupation were decreased when *A. suspensa* adults were treated orally with 0.1% of diflubenzuron for 27 d. When larvae and pupae of *A. suspensa* were treated by dipping in 0.003% - 0.1% a.i. diflubenzuron for 5 min, the incidence of crumpled wings, deformed abdomens and ovipositors in these adults was 2-7 times and 4-9 times higher than the respective controls (Lawrence, 1983).

Lofgren & Williams (1982) reported that avermectin B1, a natural product derived from the soil actinomycete *Streptomyces avermitilis*, inhibited reproduction of the queen of the red imported fire ant, *Solenopsis invicta* (Buren), when laboratory colonies were fed with avermectin B1 at concentrations as low as 0.025% in soybean oil bait. When newly emerged adult female German cockroaches, *Blattella germanica* (L.), were fed with avermectin B1 at concentrations of 6.5 mg/l and higher, 86% to 100% mortality was noted, and reproduction of survivors of these dosages was completely inhibited (Cochran, 1985). When Codling moth, *Cydia pomonella* (L.), was treated as a neonate and as a 10-d-old larvae with 0.025 mg/l or higher avermectin B1, egg production was reduced 84% to 100% (Reed et al., 1985). In the study of effects of avermectin B1 on reproduction of Mediterranean fruit fly, Oriental fruit fly, and Melon fly, Albrecht & Sherman (1987) showed that fecundity in all 3 species was significantly reduced after being treated with avermectin B1 at a dose that caused 25% female mortality. Fertility was greatly reduced in Oriental fruit fly when both male and female were treated, and in

Melon fly, fertility was significantly reduced regardless of whether treated or untreated males were paired with treated females.

When the cockroach, *B. germanica*, was treated with tunicamycin, vitellogenin was not secreted even though it did accumulate. Similar results were obtained in *G. mellonella*. In the follicle cells of dipteran insects, the export of vitellogenin was disrupted by colchicine and other microtubule inhibitors. These results suggested that these cytokeletal compounds play an important role during vitellogenin secretion (Hoffman, 1995).

Sodium tetraborate and some other boron compounds have been reported to influence reproduction of some fly species. Settepani et al. (1969) reported that when both sexes of < 24-hr-old screw-worm flies, *C. hominivorax*, were treated by feeding 1% of sodium tetraborate for 5 d, 87% of flies was killed and no oviposition at 7 d posttreatment was observed. However, Borkovec et al. (1969) reported that 1% of sodium tetraborate only slightly reduced egg hatch and pupation when newly emerged house flies, *M. domestica*, were treated by feeding for 3 d. When sodium tetraborate was increased to 2.5% and 5%, egg hatch was completely inhibited (Borkovec et al., 1969). When face fly, *M. autumnalis*, was treated with 1% boric acid by feeding for 4 d after eclosion, sterility was observed in 6 d after treatment (Lang & Treece, 1972). Mullens & Rodriguez (1992) reported that when adult *M. domestica* were fed with 1% and 2% of polybor 3 ($Na_2B_8O_{13} \cdot 4\ H_2O$) for 2 d, egg hatch was reduced 50% to 90% for 3-4 d after treatment.

Nigg and Simpson (1997) hold U.S. Patent 5,698,208 (issued Dec. 16, 1997): Use of Borax Toxicants to Control Tephritidae Fruit Fly. The unique aspect of this patent is that one feeding of sodium tetraborate toxicant leads to 7 d of no oviposition in *A. suspensa* (Nigg & Simpson, 1997).

Proteolytic Enzymes in Insects

As previously described, protein is an essential nutrition source for many insects. Digestive proteolysis is essential for the transfer of ingested protein into growth, development, and reproduction (Houseman & Thie, 1993). Most proteins taken as food by insects are macromolecular and must be processed to a form that can be absorbed for subsequent assimilation (House, 1973). Initial investigations of insect digestive proteolysis were based on methods developed for vertebrate digestive studies. As a consequence, enzyme terms similar to that of vertebrate were adopted for insects (Houseman & Thie, 1993).

General Properties and Classification of Proteinases

Proteinases involve 2 groups, endopeptidases and exopeptidases. An endopeptidase is loosely defined as a proteolytic enzyme cleaving internal peptide bonds wtih various degrees of amino acid specificity (Barrett, 1994). In most cases, endopeptidases can catalyze hydrolysis of ester and amide. Exopeptidases remove terminal amino acids from either the carboxyl end (carboxypeptidase EC 3.4.17) or the amino end (aminopeptidase EC 3.4.11) (Applebaum, 1985). Based on their mechanism of action, the endopeptidases can be divided into 4 classes: (1) serine proteinase (EC 3.4.21), e.g. chymotrypsin (EC 3.4.21.1), trypsin (EC 3.4.21.4), thrombin (EC 3.4.21.5), plasmin (EC 3.4.21.7), and elastase (EC 3.4.21.11), the active centers of which contain serine and histidine, (2) cystine proteinases (EC 3.4.22) which have a cystine in the active centre and acidic pH optima, e.g. cathepsin B (EC 3.4.22.1), papain (EC 3.4.22.2) and cathepsin L (EC 3.4.22.15), (3) aspartic proteinase (EC 3.4.23) in which 2 aspartic residues are involved in the catalytic process, e.g. pepsin (EC 3.4.23.1) and cathepsin D (EC 3.4.23.5), having a low optimum pH, and (4) metallo-proteinases (EC 3.4.24) which contain metal ions (usually zinc) at the active center (Hartley, 1960; Applebaum, 1985; Barrett, 1994). Except for metallo proteinases, the other 3 class proteinases have been reported in insects (Table 2-3).

Usually, the identification of protease type is based on hydrolysis of their specific substrates at a certain pH and on their inhibition by proteinase inhibitors exhibiting various degrees of specificity to the known vertebrate proteinases (Applebaum, 1985; Barrett, 1994). The choice of substrates for serine proteases is important when the type protease activity is being investigated. For some substrates, such as N-a-p-tosyl-L-arginine methyl ester (TAME) and N-a-benzoyl-L-arginine ethyl ester (BAEE) (for trypsin), and N-benzoyl-L-tyrosine methyl ester (BTEE) and N-acetyl-L-tyrosine ethyl ester (ATEE) (for chymotrypsin), activities have to be measured in the ultraviolet range (240-260 mm), where enzymes and crude extracts can cause interference (Sarath et al., 1990). When these esterolytic substrates are used with unpurified enzyme preparations, they may also be hydrolyzed by esterases in insects (Johnston et al., 1995).

Amidolytic substrates are more specific to proteinase activity, so, N-a-benzoyl-DL-arginine-p nitroanilide (BApNA) and N-benzoyl-L-tyrosine-p-nitronilide (BTpNA) appear to be specific substrates for trypsin and chymotrypsin, respectively (Kraut, 1977).

When different substrates are chosen to determine serine proteinase activity, pH optima will be different, since more than one enzyme exists in crude preparations, and these enzymes may hydrolyze the substrate(s) at different rates. Also, the buffer may affect the level of enzyme activity (Houseman et al., 1989; Lenz et al., 1991; Lee & Anstee, 1995). Normally, in insects the pH optima for trypsin and chymotrypsin is around pH 10.0 with amidolytic substrates (BApNA and BTpNA) and lower for esterase substrates (TAME and BTEE, around pH 8.0) (Houseman et al., 1989; Broadway, 1989; Lee & Anstee, 1995).

Proteinases in the Midgut of Insects

Since different insects have different food sources, different types of proteases have been stimulated and adapted to digest these proteins in various insects (Baker, 1981). Table 2-3 shows various proteinases determined in the digestive tract of insects by using different specific substrates and inhibitors. Serine proteinases, including trypsin and chymotrypsin, have been demonstrated as major digestive proteinases in Diptera, Lepidoptera, Orthoptera, and Coleoptera (Sharma et al., 1984; Applebaum, 1985; Christeller et al., 1992; Purcell et al., 1992). Other classes of proteinases such as cysteine proteinases and exoproteinases, etc., have also been found in Hemiptera and some Coleoptera (Murdock et al., 1987; Houseman and Thie, 1993).

For the hematophagous insects, trypsin- and chymotrypsin-like enzymes have been demonstrated to be major digestive enzymes in mosquito, *A. aegypti* (Gooding, 1973; Briegel & Lea, 1975; Graf & Briegel, 1985) and blood-sucking flies, such as, *S. calcitrans* (Champlain & Fisk, 1956; Lehane, 1977; Borovsky, 1985), and *Glossina morsitans* (Westwood) (Gooding, 1974, 1977).

In *A. aegypti,* trypsin activity, secretion and its regulation have been extensively studied (Shambaugh, 1954; Gooding, 1973; Briegel & Lee, 1975; Graf & Briegel, 1985, 1989; Barillas-Mury et al., 1995). The secretion of trypsin-like enzymes was induced by the blood meal, and the activity was correlated to the amount of blood ingestion. The same results were also found in many non-blood-feeding insects (Engelman, 1969b; Baker, 1977). Trypsin-like enzymes in the adult *A. aegypti* were multiple proteinases; the pattern of these proteinases was quite different from that in the larvae (Kunz, 1978; Graf & Briegel, 1985).

Unlike the mosquito and blood-sucking flies, the midgut of *R. prolixus* contained digestive enzymes found to be cathepsin B- and D-like proteinase, lysosomal carboxypeptide B, and aminopeptidase (Houseman, 1978; Houseman & Downe, 1980, 1983). In the predacious bugs, *Euschistus euschistoides* (Vollenhoven) (Houseman et al., 1984) and *Phymata wolffii* (Stal) (Houseman et al., 1985), these proteinases also have been found. Also, it was found in *R. prolixus*, after protease activity reached a peak that activity declined much more gradually in virgin females than in mated females (Houseman & Downe, 1983). This result may indicate that high levels of protease activity presumably lead to an increased availability of amino acids in the hemolymph for the production of yolk proteins during vitellogenesis (Persaud & Davey, 1971). In the cockroach, *Nauphoeta cinerea* (Olivier), trypsin activity in mated females increased with ovarian development and decreased after ovulation (Rao & Fisk, 1965).

In addition to blood-sucking dipteran species, serine proteases (trypsin- and chymotrypsin-like enzymes) also have been demonstrated in organic detritus-feeding flies, such as the crane fly, *Tipula abdominalis* (Say) (Sharma et al., 1984; Mahamood & Borovsky, 1992), phytophagous lepidopteran larvae such as *Manduca serta* (Johnson), *Heliothis zea* (Boddie), and *Sesamia nonagrioides* (Lef.) (Miller et al., 1974; Hamed & Attia, 1987; Lenz et al., 1991; Ortego et al., 1996), and other herbivorous insects (Knecht et al., 1974; Houseman & Thie, 1993).

In Lepidoptera, digestive endoproteinases were characterized as trypsin- and chymotrypsin-like enzymes based on their substrate specificity, pH optimum, and inhibition selectivity (Hamed & Attias, 1987; Lenz et al., 1991; Christeller et al., 1992; Lee & Ansteen, 1995). However, comparisons of insect and bovine pancreatic trypsin have detailed differences in their pH optima and their sensitivity to some protease inhibitors (Valaitis, 1995). The 2 major types of protease enzymes, trypsin- and chymotrypsin-like enzymes, in larvae of Lepidoptera had a high pH optima (about 10) which is consistent with the high pH of the midgut lumen (Ahmad et al., 1980; Johnston et al., 1991; Johnston et al., 1995). In adult Lepidoptera, trypsin optimum pH in the midgut was lower, around pH 8-9 (Eguchi et al., 1972). In addition, elastase and exoproteinases including carboxypeptidase A and B and aminopeptidase have been found in the midgut of most Lepidoptera species such as *C. obliguana* (Walker), *L. dispar*, and

S. nonagrioides (Christeller et al., 1992; Valaitis, 1995; Ortego et al., 1996). Elastase has also been reported in the black field cricket, *Teleogryllus commodus* (Walker) (Chisteller et al., 1990).

Knecht et al. (1974) reported that, in *L. migratoria*, digestive proteases have been fractionated and identified as 4 different endopeptidases on the basis of activity on casein, B-chain of oxidized insulin and several synthesized substrates. Two of them were essentially tryptic and chymotyptic and were inhibited by N-a-p-tosyl-L-lysine chloromethyl ketone (TLCK), a trypsin specific inhibitor, and N-tosyl-L-phenyl-alanine chloromethyl ketone (TPCK), a chymotrypsin specific inhibitor, respectively.

In Coleoptera, the thiol-dependent proteinases with mildly acidic pH optima (about pH 5) are commonly used as digestive enzymes. These proteinases were not blocked by the usual serine proteinase inhibitor, Diisopropyl fluorophosphate (DFP) and Phenylmethylsulphonyl fluoride (PMSF); but, were powerfully inhibited by the specific cystine proteinase inhibitor, Trans-epoxysuccinyl-L-leucylmido(4-guanidino)butane (E-64) (Kitch & Murdock, 1986; Murdock et al., 1987). In some other Coleoptera species, serine proteinases have also been found as digestive enzymes (Gooding & Huang, 1969; Baker, 1977; Houseman & Thie, 1993). Gooding & Huang (1969) reported that both trypsin- and chymotrypsin-like enzymes were detected in the gut of the predacious beetle, *Pterostichus melanarius* (Illiger), by following the hydrolysis of BAEE and BTEE. These enzymes occurred in about same concentration in both males and females. Their temperature optimum was about 47 °C and was comparable to the results reported in the cockroach, *N. cinerea* (Rao & Fisk, 1965), and mosquito, A. *aegypti* (Gooding, 1966).

Normally, endoproteinases are predominant in the midgut of insects. Baker (1982) showed that in 3 species of *Sitophilus* weevils, endopeptidase activity was found to be very low and aminopeptidase and carboxypeptidases were relatively active. These results were also found in *Rhyncosciara americana* (Terra et al., 1979), and the horn fly, *Haematobia irritans* (Linnaeus) (Hori et al., 1981), as well as some Lepidoptera, such as the corn earworm, *H. zea* (Lenz et al., 1991), and the corn stalk borer, *S. nonagrioides* (Ortego et al., 1996).

In *Apis mellifica*, 4 fractions with endopeptidase activities have been isolated and characterized from the midgut of adult worker honeybees. Fraction A was characterized as a trypsin-like enzyme. Fractions B and D had characteristics of chymotrypsin, but fraction B showed different cleavage specificity against bovine insulin B-chain, and fraction D had more splitting sites when oxidized B chain was used as a substrate compared with chymotrypsin. Fractions A and B were not detected in larval workers and queen, presumably because they consume different protein food from the adult worker (Giebel et al., 1971). Dahlmann et al. (1978) reported that fraction B was detected in the larval worker. One enzyme that hydrolyzed BApNA was also found in the larval worker, but this enzyme showed no immunological relationship with fraction A in adult.

In conclusion, proteinase types in various insects were different due to different food sources. Multiple digestive enzymes were found in all insects examined.

Proteinases in the Eggs of Insects

In addition to the midgut, proteinases have been reported also in the eggs of insects. Unlike in the midgut of insects, proteinases in insect eggs have only been investigated in *M. domestica* (Greenberg & Paretsky, 1955), *L. migratoria* (Shulov et al., 1957) *B. mori* (Kageyama et al., 1981), and *D. melanogaster* (Medina et al., 1988). The main role of these proteinases is thought to be the degradation of vitellin in embryos undergoing larval differentiation (Kuk-Meiri et al., 1966; Izumi et al., 1994).

Greenberg & Paretsky (1955) reported that proteinase in the eggs of *Musca domestica* had pH optima of 3 and 5 in hydrolyzing casein, and was recognized as cathepsin like enzyme; tryptic activity toward sodium caseinate or bovine albumin was not found in the eggs of this insect. Later, this proteinase was characterized as a cathepsin B-like proteinase, with a pH optimum of 4.5 toward N-α-carbobenzoxy-L-lysine p-nitrophenyl ester and a molecular weight 25 ± 0.5 kDa (Ribolla at al., 1993). Similarly, cathepsin B-like proteinase has also been found in *D. melanogaster* (Medina et al., 1988). This proteinase activity increases during early embryogenesis in parallel with the decrease in molecular weight (1000 kDa) of its heavy form, and decreases to low value (39 kDa) in late embryos.

In the eggs of *L. migratoria migratorioides*, two proteinases were found. One measured at pH 7.8 is probably trypsin-like; whereas the other, which is responsible for the cleavage of the casein at pH 5.6 was probably a cathepsin-like proteinase (Shulov et al., 1957). Further study showed that probably two cathepsin-like enzymes were present in eggs and that their quantitative ratio changes during development. One of these enzymes, like cathepsin B and C, is activated by mercaptoethanol and the other is not (like cathepsin A). Optimum activity of these enzymes toward hemoglobin was within the pH range 3.5-4.1 (Kuk-Meiri et al., 1966).

For the silkmoth, *Bombyx mori*, egg proteinases have been investigated in detail. Two proteinases have been reported from silkmoth eggs. One is seryl-trypsin-like proteinase which appears midway in embryogenesis, and increases steeply during completion of larval differentiation (Indrasith et al., 1988). The other is cysteine which is the major proteinase present in early developing eggs of *B. mori* (Kageyama et al., 1981; Kagayama & Takahashi, 1990). The trypsin-like serine proteinase synthesized during embryogenesis has an extremely high degree of specificity for egg specific protein (Indrasith et al., 1988), and its activity is controlled at the level of protein synthesis (Ikeda et al., 1990; Yamamoto et al., 1994). An acid cysteine proteinase was characterized as similar to the mammalian lysosomal cathepsins, especially cathepsin L (Kageyama & Takahashi, 1990). Unlike the trypsin-like proteinase, the activity and quantity of cystiene proteinase increase their level in the ovary during pupal-adult development of *B. mori*, reaching a maximum at maturation of the oocytes (Takahashi et al., 1992; Yamamota et al., 1994). This enzyme is synthesized in the follicle cells and accumulates in the oocytes (Yamamoto et al., 1994).

In addition to cathepsin-like and trypsin-like proteinases, a serine carboxypeptidase was also found in the eggs of *A. aegypti*. This proteinase was synthesized in the fat body, and then internalized by oocytes. The active form of vitellogenic carboxypeptidase is maximally present at the middle of embryonic development, and disappears by the end (Cho et al., 1991).

Insect Esterases Related to Reproduction

Esterases are a large and diverse group of enzymes, and have a wide range of substrate specificity. They are able to cleave triester phosphates, halides, esters, thioesters, amides, and peptides (Dary et al., 1990). Thus far, two esterases, JH esterase (JHE) and esterase 6 (EST 6) have been reported to be related to reproduction of insects (Richmond et al., 1980; Gilbert & Richmond, 1982; Shapiro et al., 1986; Bonning, 1997).

JHE is a carboxylesterase (EC 3.1.1.1) which hydrolyses JH. JHE is produced in both the ovary and the fat body (Shapiro et al., 1986). It is thought to play a role in JH titer regulation during the metamorphosis (McCaleb & Kumaran, 1980; Hammock, 1985; Tanaka et al., 1989) and adult reproduction cycle (Rotin et al., 1982; Renucci et al., 1984; Shapiro et al., 1986; Woodring & Sparks, 1987). JHs play a critical role in coordination of events leading to vitellogenesis in many insects. The titer of JHs in insects is regulated both by the rate of biosynthesis in the corpora allata (Tobe & Pratt, 1975) and by the rate of degradation (Hammock, 1985). Hydrolytic degradation of JHs is affected by two classes of enzymes, JHE and epoxide hydrolase (Hammock, 1985).

In female *A. ageypti*, JH III levels and JHE activity from whole body extract and hemolymph were measured. The results showed that JH III levels and JHE activity were inversely correlated after blood meal (Shapiro et al., 1986). This result was in agreement with that reported by Renucci et al. (1984). Topical application of s-benzy-o-ethylphosphoramidothiolate (BEPAT), a specific inhibitor of JHE, stopped JH hydrolysis and caused a reduction in egg hatch. These data indicated that the decline in JH levels after blood meal is at least partially a result of degradation by JHE and this decline in JH levels may be necessary to allow release of egg development neurosecretory hormone (EDNH) and 20-hydroxyecdysone for egg development (Shapiro et al., 1986). Similar results were also found in *Aedes atropalpus* by Masler et al. (1980) and Kelly et al. (1981).

Bonning et al. (1997) showed that when recombinant JHE was injected into *Acheta domesticus* (Linnaeus) on the day of the imaginal molt, ovarian development was significantly reduced, and the egg production was decreased. The result indicated that recombinant JHE is a powerful and specific anti-JH reagent and can reduce the JH below a level which is essential for egg development.

Many organophosphorus insecticides have been demonstrated to affect cholinergic nerve system in the brain by interaction with cholinesterase (Ho & Sudderuddin, 1976; Pasteur & Georghiou, 1989). Brain factors have been shown to be involved in JHE regulation (stimulation and/or inhibition) in the cabbage looper, *Trichoplusia ni* (Hübner) (Jones et al., 1981), and in *Galleria mellonella* (Linnaeus) (McCaleb & Kumaran, 1980). These results indicated that some insecticides may affect JHE through this mechanism and, subsequently, affect JH titer and egg development. Linderman et al. (1991) found that ethyl 0-4-nitrophenyloctylthiomethylphosphonate and ethyl s-phenyl octylthiomethylphosphonothioate showed potent inhibition of JHE.

Esterase 6 (EST 6) is another nonspecific carboxylesterase which can hydrolyze a wide range of substrates, possibly including some proteins (Richmond et al., 1990). This esterase was studied initially by Wright (1963) who found that the inheritance of this enzyme polymorphism is controlled by a locus (EST 6) on the third chromosome at position 36.8 in *D. melanogaster*. This enzyme was found to be a component of the male seminal fluid in *D. melanogaster* and related species (Richmond et al., 1980).

EST 6 is primarily an adult male enzyme and is transferred to the female during mating. It was detected in various digestive and other tissues throughout development, but the majority of its activity is localized to the anterior ejaculatory duct of the male reproductive tract (Sheeham et al., 1979; Healy et al., 1991). The EST 6 activity in adult males is 5 or more times greater than in any other developmental stage or in adult females. At copulation, males transfer a portion of their EST 6 into females, and they resynthesize EST 6 to virginal levels in 1-2 d (Richmond et al., 1980; Richmond & Senior, 1981).

The role of EST 6 in the reproduction of insect has been extensively studied in *D. melanogaster*. Richmond et al. (1980) showed that the seminal fluid of *D. melanogaster* males contains high activity of EST 6, and females inseminated by males having active EST 6 mated again significantly sooner than females inseminated by males without EST 6 activity. Similar results were reported on *D. melanogaster* in a series of investigations (Sheeham et al., 1979; Gilbert, 1981; Gilbert et al., 1981a). Further study showed that sperm are released from female storage organs more rapidly when the ejaculate contains more active EST 6 (Gilbert et al., 1981b).

In *D. melanogaster*, EST 6 is transferred from male to female during the first 3 min of copulation and remains active in the female reproductive tract for 1-2 hr after mating (Richmond & Senior, 1981). Meikle et al. (1990) showed that male-derived EST 6 was initially transferred into the female's reproductive tract but was translocated within minutes of the beginning of mating into the hemolymph. This enzyme was shown to be related with sperm motility in the female reproductive tract through metabolism of ejaculate lipids (Gilbert, 1981).

The presence of EST 6 in male seminal fluid has also been demonstrated to affect progeny production. When females mated with EST 6 null male (without EST 6 activity), the number of fertile eggs was reduced and malformed eggs were increased slightly (Gilbert et al., 1981; Gilbter & Richmond, 1982). However, Saad et al. (1994) reported that EST 6 activity was not associated with female remating frequency, egg production, and fertility in *D. melanogaster*.

It can be seen from above review that egg development in the reproduction of insects is a complicate process. Many factors, such as hormones including JH and ecdysteroids, neurosecretory system, ingested proteins and their digestion, and male insects etc may be involved in this process and affect egg development directly or indirectly. In this project, the effects of sodium tetraborate and imidacloprid on survival, reproduction, enzyme activities, and yolk protein synthesis of *A. suspensa* were studied. Also, the morphological changes of ovaries induced by these two chemicals were investigated.

Table 2-1. Summary of vitellogenin/or vitelline in insects.

Insect	Order/Family	Vitellogenin synthesis site	MW of vitellogenin Subunit (kDa)	Reference
Aedes aegypti (Linnaeus)	Diptera/Culicidae	FB/OV	29-155	Hagedorn & Judson, 1977 Borovsky & Whitney, 1978
Anastrepha suspensa (Loew)	Diptera/Tephritidae	OV	48	Handler & Shirk, 1986, 1988
Bombyx mori (Linnaeus)	Lepidoptera/Bombycidae	FB/or OV	30, 42, 180	Izumi et al., 1980
Caliphora erythrocephala (Meigen)	Diptera/Calliphoridae	FB/OV	46, 49, 51	Fourney et al., 1982
Ceratitis capitata (Wildemann)	Diptera/Tephritidae	FB/OV	46, 49	Rina & Mintzas, 1988
Dacus oleae (Gmelin)	Diptera/Tephritidae	FB/OV	47, 49	Levedakou & Sekeris, 1987 Zongza & Dimitriadis, 1988
Drosophila melanogaster (Meigen)	Diptera/Drosophilidae	FB/OV	44, 45, 47	Bownes & Hames, 1977 Warren & Mahowald, 1979 Mintzas & Hambysellis, 1982
Forficula auricularia (Linnaeus)	Dermaptera/Forficulidae	FB/ or OV	82, 84	Harnish & White, 1982
Hyalophora cecropia (Linnaeus)	Lepidoptera/Saturniidae	FB/OV	47	Harnish & White, 1982
Locusta migratoria	Orthoptera/Locustidae	FB	52 - 140	Chen et al., 1978 Chinzei et al., 1981
Lucilia cuprina	Diptera/Calliphoridae	FB/OV	45, 47, 49	Fourney et al., 1982
Lymantria dispar (Linnaeus)	Lepidoptera/Lymantriidae	FB/ or OV	36, 165, 190	Hiremath & Eshita, 1992
Musca domestica (Linnaeus)	Diptera/Muscidae	FB/OV	46, 48, 51, 52, 54	De Bianchi et al., 1985
Periplaneta americana (Linnaeus)	Blattodea/Blattidae	FB	59, 62	Harnish & White, 1982
Rhodnius prolixus (Stal)	Hemiptera/Reduviidae	FB/ or OV	50, 59, 160	Harnish & White, 1982
Stomoxys calcitrans (Linnaeus)	Diptera/Muscidae	OV	41, 43, 44, 47, 49, 51	Houseman & Morrison, 1986 Chen et al., 1987
Tenebrio molitor (Linnaeus)	Coleoptera/Tenebrionidae	FB/or OV	46, 49, 50	Harnish & White, 1982

FB: Fat body; MW: Molecular weight; OV: Ovary.

Table 2-2. Protein requirement and egg development in female adult insects.

Insect	Order/Family	Common name	Protein required	References
Aedes aegypti (Linnaeus)	Diptera/Culicidae	Yellow fever mosquito	Yes	Spielman, 1971; Hagedorn, 1987
Aedes albonotatus	Diptera/Culicidae	Mosquito	No	Spielman, 1971
Aedes atropalpus	Diptera/Culicidae	Mosquito	No	Wheeler & Buck, 1996
Anastrepha oblique (Macquart)	Diptera/Tephritidae	West Indian fruit fly	Yes	Braga & Zucoloto, 1981
Anastrepha suspensa (Loew)	Diptera/Tephritidae	Caribbean fruit fly	Yes	Nigg et al., 1994
Ceratitis capitata (Wiedemann)	Diptera/Tephritidae	Mediterranean fruit fly	No	Galun et al., 1981
Drosophila melanogaster (Meigen)	Diptera/Drosophilidae	Small fruit fly	Yes	Bownes & Blair, 1986; Bownes et al.,1988
Lucilia cuprina	Diptera/Calliphoridae	Sheep blow fly	Yes	Williams et al., 1977
Lucilia cuprina	Diptera/Calliphoridae	Sheep blow fly	No	Williams et al., 1977
Musca domestica (Linnaeus)	Diptera/Muscidae	House fly	Yes	Sakurai, 1978; Agui et al., 1985; Adams & Gerst, 1991
Musca domestica (Linnaeus)	Diptera/Muscidae	House fly	No	De Bianchi et al., 1985; Adams & Nelson, 1990
Musca vetustissina	Diptera/Muscidae	Bush fly	Yes	Vogt & Walker, 1987
Phormia regina (Meigen)	Diptera/Calliphoridae	Black blow fly	Yes	Pappas & Fraenkel, 1977; Yin et al., 1989
Phormia terraenovae	Diptera/Calliphoridae	Blow fly	Yes	Wilps & Zoller, 1989
Sarcophaga argyrostoma	Diptera/Sarcophagidae	Flesh fly	No	Denlinger, 1971
Sarcophaga bullata	Diptera/Sarcophagidae	Flesh fly	No	Pappas & Fraenkel, 1977
Sarcophaga bullata	Diptera/Sarcophagidae	Flesh fly	No	Huybrechts & DeLoof, 1981
Sarcophaga ruficornis	Diptera/Sarcophagidae	Flesh fly	Yes	Verma & Bisoyi, 1980
Zabrotes subfasciatus (Boheman)	Coleptera/Bruchidae	Mexican bean weevil	No	Zucoloto, 1992

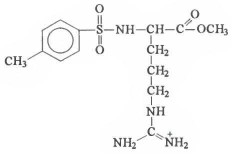
Table 2-3. The types of proteinases determined in insects.

Enzyme	Substrate	Inhibitor	Reference
General proteinase	Azocasein, Azocoll, (Azo)albumin Casein, Hemoglobin, HPA		Gooding & Huang, 1969; Engelmann, 1969; Persaud & Devey, 1971; Eguchi et al., 1972; Baker, 1977; Terra et al., 1979; Sharma et al., 1984; Borovsky, 1985; Broadway, 1989; Purcell et al., 1992; Johnston et al., 1995; Novillo et al., 1997
Serine proteinase			
Pepsin	Azocalbumin Sodium caseinate		Greenberg & Paretsky, 1955; Patterson & Fisk, 1955
Trypsin	BANA, BApNA, BAEE, TAME	Antipan, DFP, BBTI, PMSF, Chymostatin, Leupeptin, TLCK, SBTI	Rao & Fisk, 1965; Gooding, 1973; Knecht et al., 1974; Kunz, 1978; Hori et al., 1981; Baker, 1982; Sharma et al., 1984; Houseman et al, 1985; Graf & Briegel, 1985; Shukle et al., 1985; Lenz et al., 1991; Christeller et al., 1989, 1992; Valaitis, 1995; Ortego et al., 1996; Novillo et al., 1997
Chymotrypsin	APNE, ATEE, BTEE, BTpNA, GPpNA, SA2PLpNA	Antipan, DFP, BBTI, PMSF, Chymostatin TPCK	Knecht et al., 1974; Baker, 1977; Kunz, 1978; Sharma et al., 1984; Moffatt & Lehane, 1990; Lenz et al., 1991 Christeller et al., 1989, 1992; Houseman & Thie, 1993; Lee & Anstee, 1995; Ortego et al., 1996
Elastase	SA2PLpNA	PCPI	Christeller et al., 1989, 1992; Valaitis, 1995; Ortego et al., 1996;

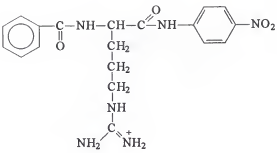
Table 2-3--continued.

Cysteine proteinase	BANA Methemoglobin	E-64, IAA; pCMB	Murdock et al., 1987; Houseman & Thie, 1993; Matsumoto et al, 1995
Cathepsin B	BANA		Houseman, 1978; Houseman & Downe, 1983; Houseman et al, 1985; Thie & Houseman, 1990
Cathepsin H	Azocasein	Oryzacystatin	Thie & Houseman, 1990; Michaud et al., 1993
Aspartic proteinase			
Cathepsin D	Hemoglobin, Methoglobin	Pepstain	Houseman & Downe, 1983; Murdock et al., 1987; Thie & Houseman, 1990; Lemos & Terra, 1991
Carboxypeptidase A	HPLA		Schneider et al., 1987; Lenz et al., 1991; Christeller et al., 1989, 1992; Ortego et al., 1996
Carboxypeptidase B	HA	IAA	Houseman & Downe, 1981, 1983; Schneider et al., 1987; Broadway, 1989; Lenz et al., 1991; Christeller et al., 1989, 1992; Ortego et al., 1996
Aminopeptidase	LPNA		Terra et al., 1979; Hori et al, 1981; Houseman & Downe, 1981, 1983; Baker, 1982; Houseman et al, 1985; Schneider et al., 1987; Lenz et al., 1991; Christeller et al., 1989, 1992; Ortego et al., 1996

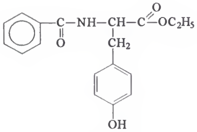
ATEE: N-acetyl-L-tyrosine ethyl ester; BAEE: N-a-benzoyl-L-arginine ethyl ester; BANA: Benzoyl-DL-arginine naphthylamine; BApNA:N-a-benzoyl-DL-arginine-p nitroanilide; BBTI: Bowman-Birk trypsin inhibitor; BTEE: N-benzoyl-L-tyrosine ethyl ester; BTpNA: N-benzoyl-L-tyrosine-p-nitronilide; DFP: Diisopropyl fluorophosphate; E-64: Trans-epoxysuccinyl-L-leucylmido(4-guanidino)butane; HA: Hippuryl-L-arginine; HPA: Hide powder azure; HPLA: Hippuryl-DL-phenyllactic acid; IAA: Iodoacetamide; LPNA: Leucine p-nitroanilide; pHMB: p-hydroxymercuribenzoate; PMSF: Phenylmethylsulphonyl fluoride; SA2PLpNA: Succinyl-Ala-Ala-Pro-Leu-p-nitroanilide; SBTI: Soybean trypsin inhibitor; TAME: N-a-p-tosyl-L-arginine methyl ester; TLCK: N-a-p-tosyl-L-lysine chloromethyl ketone; TPCK: N-tosyl-L-phenyl-alanine chloromethyl ketone.



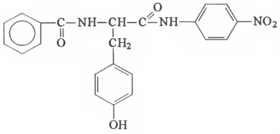
TAME



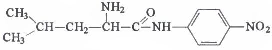
BApNA



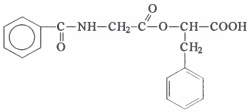
BTEE



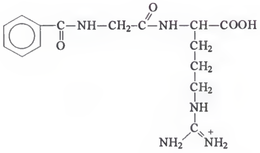
BTpNA



LPNA



HPLA



HA

Figure 2-1. Chemical structure of proteinase substrates used in this study

CHAPTER 3
EFFECT OF SODIUM TETRABORATE AND IMIDACLOPRID ON SURVIVAL AND REPRODUCTION OF *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE)

Introduction

A. suspensa infests 80 species of tropical and subtropical fruit in 23 families in Florida (Swanson & Baranowski, 1972). Because the larval stages of tephritid fruit flies develop inside fruit, the use of chemical insecticides for control of these fruit flies has focused on the adult (Budia & Vinuela, 1996). A common method to control adult *A. suspensa* is a bait spray containing malathion (Calkins, 1993; Simpson, 1993), although alternative methods have been used to control adults of this fruit fly, such as the sterile male technique (Holler & Harris, 1993). As application of malathion over wide areas has resulted in environmental concerns and public protests, interest has shifted to compounds with low toxicity, including boron compounds.

Azadirachtin and benzyl-1,3-benzodioxole derivatives (BBDs), obtained from the neem tree, *Azadirachta indica* A. Juss (Meliaceae), and Panamanian hard wood, *Dalbergia retusa* (Henley), respectively, have been reported to affect fecundity and fertility of fruit flies (Cha42ng et al., 1988, 1994; Song et al., 1990; Stark et al., 1990; Dong et al., 1997; Di Ilio et al., 1999). Sodium tetraborate and other boron compounds may act as larvicides, adulticides, or sterilants for termites (Grace, 1991), cockroaches

(Bare, 1945; Cochran, 1995), ants (Klotz et al., 1997), and flies (Settepani et al., 1969; Lang & Treece, 1972; Mullens & Rodriguez, 1992; Hogsette & Koehler, 1994). One advantage of sodium tetraborate in pest control is its relatively low toxicity to mammals with oral LD_{50} of 4500 and 4980 mg/kg for male and female Sprague-Dawley rats (Weir & Fisher, 1972). Sodium tetraborate has been registered with the U.S. EPA as an active ingredient for pesticidal formulation (Borax Europe limited, Guildford, UK).

Settepani et al. (1969) reported that when both sexes of < 24-hr-old screw-worm flies, *C. hominivorax*, fed on food containing 1% sodium tetraborate for 5 d, 87% of flies were killed and no oviposition at 7 d posttreatment was observed. Borkovec et al. (1969) reported that 1% sodium tetraborate slightly reduced egg hatch and pupation when newly emerged house flies, *M. domestica*, were treated by feeding for 3 d. When the sodium tetraborate concentration was increased to 2.5% and 5%, egg hatch was completely inhibited. Nigg & Simpson (1997) has showed that oviposition of *A. suspensa* was delayed up to 7 d after one feeding with sodium tetraborate.

Imidacloprid is a new chloronicotinyl insecticide which has been found to be effective against many economically important insect pests such as aphids, whiteflies, and beetles (Palumbo et al., 1996; Boiteau et al., 1997)

In this study, the toxicity of sodium tetraborate and imidacloprid to *A. suspensa* and their effects on the mortality, fecundity, and fertility of adult *A. suspensa* were investigated.

Materials and Methods

A. suspensa were obtained as 9-d-old pupae from Florida Department of Agricultural and Consumer Services, Division of Plant Industry, Gainesville, FL. Male and female flies which emerged over 4 hr were placed in separate cages (30 x 30 x 30 cm) with a stocking access front (BioQuip, Gardena, CA). Flies were held in the laboratory at 25 to 28 °C and 50 to 70% RH, with a photoperiod of L:D 12:12.

Normal food was supplied as a yeast/sugar (1:3, w/w) patty for food and a 1% agar patty for water. Food with sodium tetraborate or imidacloprid was prepared by adding the appropriate amount of sodium tetraborate or imidacloprid to a mixture of sucrose, yeast hydrolysate enzymatic (ICN Biomedicals, Inc., Aurora, OH), and agar (Fisher Scientific, Fair Lawn, NJ) (10:2:0.5, w/w/w) dissolved in 5 volumes of distilled water (sugar:DDW, 1:5 w/v). Egg laying squares were prepared by dipping 4 x 4 cm double cheesecloth squares into a warm solution of petroleum jelly (EPACT Corp., Brooklyn, NY), paraffin gulf wax (Boyle-Midway, Inc., New York, NY), and candle color dye (C-9 Holiday Red, Walnut Hill Co., Bristol, PA) (1:2:0.05, w/w/w) for a few seconds, transferring into cool tap water, and drying at room temperature.

Three experiments including preliminary experiment, full experiment, and discrimination experiment were conducted in this sub-project.

Part I: Preliminary Experiment

Four experiments were carried out to examine toxicity of sodium tetraborate and imidacloprid to *A. suspensa* and the effect of different concentrations of these chemicals on the fecundity and fertility of *A. suspensa* treated at different ages.

Experiment 1

The purposes of experiment 1 were to observe the oviposition behavior and pattern of female *A. suspensa*. Twenty-five 950 cm^3 transparent plastic containers were prepared. One newly emerged male fly and one newly emerged female fly were placed in each container which was covered with a net cap (10.5 cm diameter). These flies were supplied with yeast/sugar patty for food and a 1% agar patty for water and one egg laying square was placed on the screen top of each container. The egg production of each female fly was recorded daily when oviposition was started. Dead males were replaced with live males during the experiment. The number of eggs was recorded continuously for each fly until the female fly was dead.

Experiment 2

The purpose of experiment 2 was to assess the effect of concentration on the acute toxicity of sodium tetraborate and imidacloprid to *A. suspensa*. Thirty-six 950 cm^3 transparent plastic containers were prepared. Five paired newly emerged flies were transferred to each container and fed immediately with food containing different concentrations of sodium tetraborate or imidacloprid (Nigg et al., 1994). Six dose levels of sodium tetraborate, 0, 0.5, 1.0, 2.0, 3.0, and 4.0%, and imidacloprid, 0, 1.0, 2.0, 4.0, 6.0, and 8.0 mg/l, were tested; each dose level had 3 replicates. After 24 hr treatment, the food with pesticide was removed and changed to normal food. Dead males and females from each container were counted and recorded daily until the experiment was stopped on day 14 after treatment.

Experiment 3

The purpose of experiment 3 was to determine the effect of various concentrations of sodium tetraborate and imidacloprid on egg production and egg hatch of *A. suspensa* treated for different periods. Six groups of 5 pairs of newly emerged flies were set up as described above. Each group had 6 containers. Three groups were treated with 0, 0.02, 0.05, 0.1, 0.2, and 0.5% sodium tetraborate by feeding 24, 48, and 168 hr; another 3 groups were treated with 0, 0.05, 0.1, 0.2, 0.5, and 1.0 mg/l imidacloprid as above. Each treatment was replicated 3 times. Control food and food with sodium tetraborate or imidacloprid were prepared and changed daily. After the treatment period, food was removed and changed to normal food. The 1% agar paddy for water was changed every other day.

Experiment 4

The purpose of experiment 4 was to determine the effects of 0.5% sodium tetraborate and 1.0 mg/l imidacloprid on the egg production and egg hatch of *A. suspensa* treated at different ages or as individual sexes. Two groups of 5 pairs of newly emerged flies were set up as previously described. One group was treated with 0.5% sodium tetraborate at day 0, 1, 3, and 5 after emergence and another group with 1.0 mg/l imidacloprid by feeding 24 hr; 24 hr later, food was changed to normal food. For single sex fly treatment, 2 groups of 5 newly emerged male or female flies were set up into separate test containers, treated immediately by feeding food containing 0.5% sodium tetraborate or 1.0 mg/l imidacloprid. After 24 hr, 5 untreated female or male flies of the same age as the treated flies were placed with the opposite sex, and food with pesticide was removed and changed to the normal food. Controls were prepared the same as treatment except for normal food without sodium tetraborate or imidacloprid.

To determine the effects of sodium tetraborate and imidacloprid on the fecundity and fertility of *A. suspensa* treated with different concentrations or treated at different ages, dead female flies in each container were recorded daily for 14 d after feeding food with sodium tetraborate or imidacloprid to the experiment was terminated.

During the oviposition period, eggs from each container were counted and recorded daily at 8:00 am. A clean egg laying square was then placed on the screen top of each container and covered with a small petri dish containing a piece of wetted tissue paper. At 3:00 pm, eggs laid on the covered squares were counted and rinsed into a small beaker with distilled water, transferred to black filter paper in a 9 cm diameter petri dish, and wetted with 0.07% acidified sodium benzoate (4.2 ml of 10% HCl in 1 liter solution). The petri dishes were sealed with para-film, and kept in 30 °C incubator. The number of hatched larvae in each petri dish was checked and recorded daily for the next 7 d. Egg production was monitored continuously from the first oviposition day to day 14 after emergence when experiments were stopped. Fecundity was calculated as eggs per female per day, and fertility was calculated as percent egg hatch.

Part II: Full Experiment

Two experiments were carried out to assess the effects of feeding different periods of various concentrations of sodium tetraborate on survival and reproduction of *A. suspensa* treated as a newly emerged adult or as a 10-d-old adult. In the first experiment, 3 groups of 50 containers each were prepared. Five pairs of newly emerged flies were placed in each container and treated immediately with 0.02, 0.05, 0.1, 0.2, and 0.5% sodium tetraborate by feeding 24, 48, and 168 hr. Each dose level had a matched control; controls and treatments at each dose level were replicated 5 times. In the second experiment, another 3 groups of containers with 5 pairs of flies from same population as above experiment were treated at age 10 days as described above.

Dead flies in each container were recorded daily by sex after feeding food with sodium tetraborate and continued for 20 d until the experiment was terminated. At the end of each experiment, all flies were killed and total male and female flies in each container were recorded. During the oviposition period, eggs from each container were counted, recorded, and set up for egg hatch as described previously.

Part III: Discrimination Experiment

Two experiments were conducted to determine if flies are repelled by sodium tetraborate by comparing mortality, fecundity, and fertility of *A. suspensa* by allowing flies a choice or no choice of food containing sodium tetraborate. In the first experiment, 2 groups of containers with 5 pairs of newly emerged flies in each container were treated immediately with 0.1% to 1.0% sodium tetraborate by feeding for one week, one group was fed only on food containing sodium tetraborate, termed no-choice food. The second group was offered control food without sodium tetraborate and food containing sodium tetraborate, termed choice food.

According to previous experimental results, the minimum concentration of sodium tetraborate which reduced the fecundity of *A. suspensa* after one week feeding was 0.1%. In these 2 experiments, 4 levels of sodium tetraborate, 0.1, 0.2, 0.5, and 1%, were used to feed flies; each level had 5 replicates. Five containers in which flies were fed only on control food without sodium tetraborate were used as the controls. One egg laying square was placed on the screen top of each container. Control food and food with sodium tetraborate were prepared and changed daily. After one week of feeding, both control food and pesticide food were removed and changed to normal food. A 1% agar patty for water was changed every other day. In the second experiment, 2 groups of 5 paired flies from the same population as above experiment were set up at day 8, fed with normal food, and treated on day 10 as described above. Dead flies, number of eggs, and percent of egg hatch were recorded daily as described above.

Statistical Analysis

Data were analyzed with the general linear model (GLM) procedure (SAS Institute, 1989). Significant differences among control and treatments ($P < 0.05$) were tested by Tukey's honest significant difference (HSD) test (SAS Institute, 1989). LC_{50} and LT_{50}s were estimated by probit analysis (SAS Institute, 1989). Significant differences were identified by failure of 95% confidence intervals (CI) to overlap.

Results and Discussion

Part I: Preliminary Experiment

Oviposition pattern of *A. suspensa*

Oviposition of *A. suspensa* fed on yeast/sugar (1:3, w/w) patty for food and a 1% agar patty for water was observed and recorded for 90 d after emergence (Fig. 3-1). Oviposition began at 6 to 7 d after emergence as an adult which agrees with the results reported by Lawrence (1989). When there were not suitable oviposition sites, oviposition of *A. suspensa* was suspended for at least 5 d. Except for a few females which laid some eggs on the wall or bottom of containers, females oviposited only on egg laying square. In the first few oviposition days, the average number of eggs per day was around 15 eggs per female. On day 9 to 10 after emergence, egg production increased to >25 eggs per female per day and this oviposition rate was continuous about 10 more days. When flies were around 30 d old, the oviposition rate decreased gradually as fly age increased. These results were similar to but with some differences from the results reported by Lawrence (1983, 1989). Lawrence reported that flies with a high oviposition rate were 10 to 17 d old and egg production decreased greatly after 20 d old, which is around 10 d shorter than our results. This difference may be due to a different food source, different genetics (population), or other rearing conditions. According to Lawrence (1989), the oviposition pattern and fecundity of *A. suspensa* were different when adults of *A. suspensa* were fed on different diets. Sivinski (1993) reported that the average of oviposition rate of domestic female *A. suspensa* kept in colony for more than 15 years was 3.8 eggs per day over 55 d of lifespan, while wild females produced 1.9 eggs per day over 74 d of lifespan.

Toxicity of sodium tetraborate and imidacloprid to *A. suspensa*

LC_{50}s 48 hr after treatment with sodium tetraborate for male and female *A. suspensa* were significantly different (2.6% and 4.4%, respectively). The imidacloprid LC_{50} 48 hr for males was lower than females, but the difference was not significant (Table 3-1). Mortality of both male and female flies increased with increasing dose levels of both chemicals; male mortality was higher than female in the treatments with the same dosage level (Tables 3-2 and 3-3). LT_{50}s estimated for sodium tetraborate and imidacloprid decreased with increasing dose levels over 24 hr treatment period in both male and female flies; female flies had a higher LT_{50} than the male (Tables 3-4 and 3-5). These results indicate that mortality in both male and female flies was concentration dependent and that male flies were more sensitive to both sodium tetraborate and imidacloprid than females.

Effects of sodium tetraborate and imidacloprid on fecundity and fertility of *A. suspensa*

Fecundity of *A. suspensa* was not affected by less than 0.2% concentrations when flies were treated as newly emerged adult with 0.02% to 0.5% sodium tetraborate by feeding 24 hr, and it was reduced significantly only by 0.5% sodium tetraborate (Table3-6). If feeding with sodium tetraborate was continuous for 48 hr or 168 hr after eclosion, most tested concentrations of sodium tetraborate caused a reduction of egg production; higher concentrations caused a greater reduction. All flies died before oviposition began after feeding on food with 0.2% and 0.5% sodium tetraborate in the 168 hr treatment. The reduction in egg production was also proportional to the sodium tetraborate feeding time at each dosage level. These results agree with Budia & Vinuela (1996) and Diaz et al. (1996) who treated other tephritid species with cyromazine. They reported that the fecundity of *C. capitata* and *A. obliqua,* was reduced only by higher (≥500 mg/l) concentrations when flies were treated orally with 10-1000 mg/l cyromazine..

Similar to the fecundity results, fertility of flies treated with sodium tetraborate by feeding 24 hr was not affected by ≤ 0.1% concentrations, but was reduced with higher concentrations (0.2 and 0.5%) (Table 3-6). Fecundity of flies treated with 0.2% sodium tetraborate was not different from controls, but the fertility was affected. This result may indicate that dosage of sodium tetraborate affecting male reproduction seems to be lower than that affecting female reproduction of *A. suspensa*, or fertility was affected greater than fecundity in *A. suspensa* by sodium tetraborate; however more data are needed to support this conclusion. In contrast to sodium tetraborate, cyromazine affected fecundity more than fertility in *C. capitata* (Budia & Vinuela, 1996).

Fecundity of *A. suspensa* was reduced dramatically regardless of fly age when both male and female flies were exposed to 0.5% sodium tetraborate by feeding 24 hr (Table 3-7). When only female flies were treated immediately after emergence with 0.5% sodium tetraborate by feeding for 24 hr, fecundity was reduced significantly. However, if males only were treated as above, fecundity was not reduced, but fertility was affected. Similar results were also found in another Tephritidae species, *A. ludens* treated with cyromazine (Moreno et al., 1994). They reported that fecundity of *A. ludens* was significantly reduced when young adult females were fed with cyromazine at 0.1- 0.5% concentrations; but no effect was found when only male flies were treated. These data may indicate that unlike *Drosophila* species (Herndon & Wolfner, 1995; Boake & Moore, 1996), male flies do not affect egg production in Tephritidae species.

Unlike the fecundity results, the fertilities of untreated females paired with treated males and the fertility of treated females paired with untreated males were reduced about 50% and 30%, respectively; reduction of fertility was greater when males were treated. When both male and female flies were treated with 0.5% sodium tetraborate by feeding 24 hr, regardless of fly age prior to mating, fertility was reduced significantly. Similar results were found by Alberecht & Sherman (1987) on other fruit fly species treated with avermectin B1. The results showed that the fertility in *D. cucurbitae* was reduced above 50% when both male and female flies were treated, and in *D. dorsalis*, fertility from both groups was reduced significantly regardless of whether treated or untreated males were paired with treated females.

When flies were treated immediately after emergence with 0.05 to 1.0 mg/l imidacloprid by feeding for 24 hr, fecundity from all treatments was not different from controls (Table 3-8). However, when treatments were continuous for 48 hr, fecundity was affected with 0.5 mg/l or higher concentration, In the 168 hr treatment, egg production was reduced significantly when flies were fed with 0.2 mg/l or higher concentrations of imidacloprid.

Fertility was not affected by most tested concentrations of imidacloprid when flies were treated for 168 hr; 0.2 mg/l, however, reduced fertility of *A suspensa*. This result may be explained as that after flies were treated with 0.2 mg/l concentration, the male fertility was affected when oviposition and mating occurred; for flies treated with higher concentrations, since oviposition was delayed, male fertility (here means mating capacity) had recovered to a normal level when female began oviposition again. De Cock et al. (1996) reported that oviposition and egg hatch were not affected when mature adults of *Podisus maculiventris* were treated with a sublethal concentration (0.01 mg/l) of imidacloprid for 2 weeks. Unlike 0.5% of sodium tetraborate, 1.0 mg/l of imidacloprid did not affect either fecundity or fertility, whether flies were treated at different ages or treated as single or both sexes by feeding for 24 hr (Table 3-9).

Part II: Full Experiment

Mortality

When flies were treated with sodium tetraborate as newly emerged adults for 24 hr, mortality of both male and female flies fed on various dose levels ranging from 0.02% to 0.5% was not different from controls (Figs. 3-2A and 3-3A). If the treatment period was increased to 48 hr, there was mortality in the 0.5% treatment for both males and females; female mortality was less than male mortality. Concentrations lower than 0.5% did not cause mortalities different from control (Figs. 3-2B and 3-3B). When flies were treated over 168 hr, both male and female flies were killed by 0.5% sodium tetraborate at day 6 after treatment; 0.2% sodium tetraborate also caused mortalities with 50% (male) and 40% (female) at day 7, and 100% and 96% at day 20 after treatment for male and female, respectively; 0.1% or lower concentrations did not increase mortality compared with control (Figs. 3-2C and 3-3C).

When flies were treated as 10-d-old adults, a similar mortality pattern was found. Except for 0.5% in the 48 hr treatment which caused mortality in both male and female significantly different from control, other concentrations did not affect mortality in 24 hr and 48 hr treatment (Figs. 3-4 and 3-5, A & B). When the treatment period was 168 hr, both male and female mortality were increased significantly after flies were treated by feeding 0.2% and 0.5% sodium tetraborate (Figs. 3-4C and 3-5C).

These results indicate that mortalities in both male and female flies were concentration and treatment period dependent; higher concentration and longer treatment periods caused higher mortality regardless of fly age. However, when flies were treated with 0.5% sodium tetraborate for 48 hr or 0.2% and 0.5% sodium tetraborate for 168 hr, mortality in newly emerged flies was higher than that in 10-d-old flies (Figs. 3-2-3-5; B and C). These observations indicate that newly emerged flies were more sensitive to sodium tetraborate than 10-d-old flies.

Fecundity

Similar to the results in the preliminary experiment, when flies were treated as newly emerged adult with various concentrations of sodium tetraborate for 24 hr, only 0.5% sodium tetraborate reduced fecundity significantly (Table 3-10). Fecundity in flies treated for 48 hr was also reduced significantly only by 0.5% sodium tetraborate; this result is different from the result in preliminary experiment in which fecundity was affected by feeding on both 0.2% and 0.5% sodium tetraborate for 48 hr. When treatment was continued over 168 hr, 0.1% sodium tetraborate caused egg production to decrease significantly, and higher concentrations stopped oviposition of survivors for 20 d after treatment. Reduction in egg production was greater in the first week after treatment than in the second week after treatment (Fig. 3-6). As in the newly emerged fly treatment, when flies were treated as 10-d-old adults, fecundity in the first week after treatment was reduced about 70% only by 0.5% concentration in both 24 hr and 48 hr treatment. In the 168 hr treatment, 0.1% and 0.2% sodium tetraborate caused fecundity to decrease significantly, especially in the first 10 d after treatment. A concentration of 0.5% reduced egg production in the first few days after treatment, and then stopped oviposition until all flies died (Table 3-11, Fig. 3-7).

Fertility

Regardless of flies being treated as newly emerged adults or 10-d-old adults, egg hatch in the 24 hr and 48 hr treatments was not affected by low concentrations; 0.5% sodium tetraborate reduced fertility from 80% to 50% and 60% for the newly emerged fly and 10-d-old fly treatment, respectively. When flies were treated over 168 hr, egg hatch was reduced 60% by 0.1% sodium tetraborate in the newly emerged fly treatment and higher concentrations stopped oviposition or killed all flies; when flies were treated as 10-d-old adults for 168 hr, 0.1% or lower concentrations did not affect egg hatch, and 0.2% and 0.5% sodium tetraborate significantly reduced egg hatch by 40% and 70%, respectively, for 20 d after treatment (Table 3-12).

Part III: Discrimination Experiment

Mortality

Similar to the results in the first 2 part experiments, mortality in both male and female flies was directly related to concentrations of sodium tetraborate in the no choice test, and male mortality was higher than female mortality in the first few days after treatment regardless of fly age (Figs. 3-8 and 3-9, B and D). When flies were fed 0.2% or higher concentrations of sodium tetraborate, almost all males and females were killed after 7 d of feeding. LT_{50}s estimated for female flies were higher than for males within the same dosage level for both newly emerged flies and 10-d-old flies being treated (Tables 3-13 and 3-14).

In the choice test, mortality patterns in both males and females were similar to that found in the no choice test, that is, mortality was concentration dependent and male mortality was higher than female. LT_{50}s estimated for both males and females in the choice test were about the same as that of half concentration of sodium tetraborate in the no choice test, e.g., flies given a choice between control food and 1.0% sodium tetraborate food took as long to die as flies exposed to 0.5% sodium tetraborate alone (Tables 3-13 and 3-14). These results indicated that $\leq 1.0\%$ sodium tetraborate did not repel flies and flies randomly chose between control food and sodium tetraborate food for feeding. Comparable results were reported by Hogsette & Koehler (1994) who worked on house flies, *M. domestica*, using other boron compounds, boric acid, and polybor 3 (disodium octaborate tetrahydrate). They found that low concentrations of these 2 chemicals did not repel flies, but when concentration was increased to above 2.25% for boric acid and 3% for polybor 3, flies appeared to be repelled. Similar results were also found in *B. germanica* (Strong et al., 1993).

Fecundity

Fecundity was significantly reduced for 14 days when flies were treated as newly emerged adult by feeding on no choice food containing 0.1% sodium tetraborate (Table 3-15, Fig. 3-10A). When the concentration was increased to 0.2% or higher, 95% flies were killed before oviposition began and no eggs were oviposited by survivors. In the choice test, fecundity was not affected when flies were treated with 0.1% and 0.2% sodium tetraborate; 0.5% and 1% sodium tetraborate killed most flies after 7 d of feeding, and no oviposition was observed for survivors for 20 d after treatment (Table 3-15, Fig. 3-10B).

Similar results were also observed in the10-d-old fly treatment. That is, 0.1% sodium tetraborate in the no-choice test significantly reduced egg production for 20 d after treatment; higher concentrations stopped oviposition of survivors for 14 d after treatment

(Fig. 3-10C). In the choice test, 0.5% and 1% sodium tetraborate reduced egg production from 12 to 2.5 and 2.0 eggs per day, respectively, in the first few days after treatment and stopped oviposition of survivors for the next 14 d after 7 d of treatment (Table 3-16, Fig. 3-10D). These results showed that flies did not discriminate between control food and food containing sodium tetraborate at up to 1.0% concentration.

Fertility

Similar to fecundity results, fertility was significantly reduced when flies were treated as newly emerged adults by feeding one week on 0.1% or higher concentrations of sodium tetraborate in the no-choice test; in the choice test, egg hatch was reduced 25% or higher by feeding 0.2% or higher concentrations of sodium tetraborate (Table 3-17). Unlike newly emerged flies, egg hatch in the no-choice test was not affected by 0.1% sodium tetraborate when flies were treated as 10-d-old adults. When concentration was increased to 0.2% or higher, egg hatch was reduced significantly (60- 87%) and higher concentration caused greater reductions. Similarly, in the choice test, 0.2% sodium tetraborate did not affect egg hatch when flies were treated as 10-d-old adult, 0.5% and 1% sodium tetraborate significantly reduced egg hatch after one week treatment (Table 3-18). These results indicated that *A. suspensa* did not discriminate between food with or without ≤ 1% sodium tetraborate for feeding.

From the results of these experiments, it can be concluded that toxicity of sodium tetraborate and imidacloprid to *A. suspensa* was concentration dependent with males more sensitive than females. The concentrations of sodium tetraborate and imidacloprid at which less than 10% of flies were killed after 24hr feeding were 0.5% and 1.0mg/l, respectively.

Fecundity and fertility were not affected when flies were treated with ≤1.0mg/l imidacloprid by feeding 24hr regardless of fly age. However, for sodium tetraborate treated flies, both fecundity and fertility were reduced significantly when flies were fed with 0.5% sodium tetraborate for 24hr regardless of fly age. When the treatment was continued to 48hr, 0.5% sodium tetraborate increased the mortality of both male and female significantly; fecundity and fertility were reduced with greater reduction than in flies treated for 24hr as newly emerged or 10-d-old adults. When the treatment was 168hr, the concentration of sodium tetraborate at which fecundity and fertility were reduced was 0.1%; higher concentrations killed 95% of the flies and stopped oviposition of survivors for 14 days after 168hr treatment. When flies were fed with 1.0% sodium tetraborate, flies were not repelled by sodium tetraborate and they randomly chose between control food and sodium tetraborate food for feeding when both food types were supplied.

The general effects of sodium tetraborate and imidacloprid on survival and reproduction of *A. suspensa* have been described. The next chapter will focus on the morphological effects of these two chemicals on ovary development.

Table 3-1. LC_{50} s [a] of sodium tetraborate and imidacloprid to *A. suspensa* treated as newly emerged adult by feeding 24 hr (48 hr mortality).

	Male		Female	
Pesticide	LC_{50} (95% CI)	Slope ± SE	LC_{50} (95% CI)	Slope ± SE
Sodium tetraborate[a]	2.59 (2.15 - 3.04)	6.25 ± 2.30	4.42 (3.47 -14.58)	4.30 ± 2.73
Imidacloprid[b]	4.46 (3.57 - 5.45)	4.48 ± 0.91	5.92 (4.84 - 7.63)	4.58 ± 1.44

[a] LC_{50} was expressed as percent (w/v).

[b] LC_{50} was expressed as mg/l.

Table 3-2. Mean mortality of *A. suspensa* treated for 24 hr as newly emerged adults with sodium tetraborate.

	Mortality (%) 48 hr after treatment		Mortality (%) 168 hr after treatment	
Conc. (%)	♂	♀	♂	♀
0	0	0	6.7 ± 11.5	0
0.5	0	0	13.3 ± 11.5	6.7 ± 11.5
1.0	0	0	60.0 ± 40.0	13.3 ± 11.5
2.0	20.0 ± 34.6	6.7 ± 11.5	100	100
3.0	80.0 ± 0.0	26.7 ± 11.5	100	100
4.0	80.0 ± 20.0	40.0 ± 20.0	100	100

Table 3-3. Mean mortality of *A. suspensa* treated for 24 hr as newly emerged adults with imidacloprid.

	Mortality (%) 48 hr after treatment		Mortality (%) 168 hr after treatment	
Conc. (mg/l)	♂	♀	♂	♀
0	0	0	13.3 ± 11.5	6.7 ± 11.5
1.0	0	0	33.3 ± 23.1	0
2.0	6.7 ± 11.5	0	40.0 ± 20.0	20.0 ± 20.0
4.0	40.0 ± 20.0	33.3 ± 41.6	100	93.3 ± 11.5
6.0	73.3 ± 11.5	33.3 ± 11.5	100	100
8.0	86.7 ± 11.5	80.0 ± 20.0	100	100

Table 3-4. LT_{50} s[a] of sodium tetraborate to *A. suspensa* treated as newly emerged adult by feeding 24 hr.

	Male		Female	
Conc. (%)	LT_{50} (95% CI)	Slope ± SE	LT_{50} (95% CI)	Slope ± SE
1.0	5.12 (4.60 – 5.72)	7.60 ± 2.34	7.68 (6.93 – 9.57)	8.28 ± 5.05
2.0	2.50 (2.07 – 2.89)	6.33 ± 1.68	3.58 (3.09 – 4.19)	6.22 ± 2.11
3.0	1.73 (1.40 – 2.02)	8.23 ± 3.97	2.39 (1.95 – 3.11)	5.62 ± 2.83
4.0	1.61 (1.29 – 1.92)	6.61 ± 2.19	2.31 (1.97 –2.66)	9.88 ± 8.86

[a] LT_{50} was expressed as day.

Table 3-5. LT_{50} s [a] of imidacloprid to *A. suspensa* treated as newly emerged adult by feeding 24 hr.

Conc. (mg/l)	Male		Female	
	LT_{50} (95% CI)	Slope ± SE	LT_{50} (95% CI)	Slope ± SE
2.0	> 14		> 14	
4.0	2.05 (1.56 – 2.56)	3.94 ± 0.84	2.71 (2.20 – 3.40)	4.56 ± 1.31
6.0	1.40 (0.98 – 1.77)	4.36 ± 1.35	2.20 (1.76 – 2.84)	4.99 ± 1.93
8.0	1.14 (0.62 – 1.48)	3.92 ± 1.43	1.27 (0.81 – 1.62)	4.10 ± 1.37

[a] LT_{50} was expressed as day.

Table 3-6. Mean fecundity and fertility of *A. suspensa* treated as newly emerged adult with various concentrations of sodium tetraborate by feeding different periods.

	Fecundity (eggs/female/day)			Fertility [a]
Conc. (%)	24 hr treatment	48 hr treatment	1 wk treatment	(% egg hatch)
0	21.8 ± 6.8 a	14.9 ± 1.4 a	18.0 ± 1.6 a	79.2 ± 6.9 a
0.02	19.2 ± 3.0 ab	10.9 ± 5.6 ab	18.2 ± 3.4 a	80.9 ± 2.5 a
0.05	18.9 ± 3.8 ab	9.8 ± 1.9 ab	7.0 ± 4.1 b	76.2 ± 8.8 a
0.1	15.5 ± 5.6 ab	8.0 ± 3.7 ab	2.8 ± 0.9 b	66.7 ± 20.2 ab
0.2	19.1 ± 4.9 a	4.3 ± 3.0 bc	- [b]	27.6 ± 13.4 bc
0.5	7.8 ± 4.2 b	1.3 ± 1.6 c	- [b]	29.4 ± 2.8 c

[a] Data were from 24 hr treatment.

[b] All flies died before oviposition began.

Means in each column followed by the same letter are not significantly different, (P = 0.05, Tukey's HSD test).

Table 3-7. Mean fecundity and fertility of *A. suspensa* treated as different age adult with 0.5% sodium tetraborate by feeding 24 hr.

Treatment age (day)	Treated sex	Fecundity (eggs/female/day)	Fertility (% egg hatch)
0	Control	17.9 ± 3.8 a	78.1 ± 10.7 a
0	M	17.2 ± 2.8 a	36.5 ± 16.8 b
0	F	7.7 ± 4.4 b	53.2 ± 34.8 ab
0	M&F	3.2 ± 3.8 b	29.4 ± 2.8 b
1	M&F	3.0 ± 0.6 b	13.8 ± 20.9 b
3	M&F	2.1 ± 1.9 b	15.7 ± 9.4 b
5	M&F	2.4 ± 1.4 b	18.2 ± 8.0 b

Means in each column followed by the same letter are not significantly different, (P = 0.05, Tukey's HSD test).

Table 3-8. Mean fecundity and fertility of *A. suspensa* treated as newly emerged adult with various concentrations of imidacloprid by feeding different periods.

Conc. (mg/l)	Fecundity (eggs/female/day) 24 hr treatment	48 hr treatment	1 wk treatment	Fertility [a] (% egg hatch)
0	14.3 ± 2.7a	20.4 ± 2.3a	21.0 ± 1.6a	92.7 ± 2.0a
0.05	12.2 ± 2.3a	16.8 ± 3.7ab	20.1 ± 0.2a	83.6 ± 12.2ab
0.1	13.3 ± 3.8a	14.4 ± 2.8ab	15.6 ± 4.1ab	71.0 ± 13.4ab
0.2	12.7 ± 3.3a	14.2 ± 0.6ab	11.9 ± 0.7bc	55.4 ± 23.6b
0.5	10.5 ± 0.5a	10.6 ± 2.2bc	5.4 ± 0.8c	82.9 ± 4.5ab
1.0	9.7 ± 4.2 a	6.7 ± 0.3c	5.9 ± 1.1c	91.1 ± 3.2ab

[a] Data were from 168 hr treatment.

Means in each column followed by the same letter are not significantly different, (P = 0.05, Tukey's HSD test).

Table 3-9. Mean fecundity and fertility of *A. suspensa* treated as different age adult with 1.0 mg/l imidacloprid by feeding 24 hr.

Treatment age (day)	Treatment sex	Fecundity (eggs/female/day)	Fertility (% egg hatch)
0	Control	14.4 ± 1.0 a	82.5 ± 11.5 a
0	M	12.4 ± 2.1 a	81.4 ± 12.8 a
0	F	10.5 ± 0.4 a	78.2 ± 14.7 a
0	M&F	8.9 ± 4.6 a	84.1 ± 4.0 a
1	M&F	11.3 ± 1.7 a	77.0 ± 6.1 a
3	M&F	9.5 ± 0.5 a	79.7 ± 12.8 a
5	M&F	8.2 ± 1.9 a	75.5 ± 19.5 a

Means in each column followed by the same letter are not significantly different, (P = 0.05, Tukey’s HSD test).

Table 3-10. Mean fecundity of *A. suspensa* treated as newly emerged flies with various concentrations of sodium tetraborate by feeding different periods.

	Eggs/female/day (Mean ± SD)					
	Day 7-13 after treatment			Day 7-20 after treatment		
Conc. (%)	24 hr treatment	48 hr treatment	168 hr treatment	24 hr treatment	48 hr treatment	168 hr treatment
0	6.0 ± 4.0a	6.7 ± 4.1a	6.9 ± 4.0a	9.0 ± 3.6a	9.8 ± 2.5a	9.9 ± 4.4a
0.02	5.0 ± 4.3a	4.4 ± 2.9ab	6.3 ± 6.1a	7.8 ± 3.5a	8.2 ± 1.8ab	10.4 ± 4.9a
0.05	6.1 ± 6.5a	9.1 ± 5.6a	7.3 ± 2.9a	9.8 ± 5.7a	11.6 ± 4.7a	9.2 ± 1.9a
0.1	7.0 ± 4.9a	5.5 ± 2.7a	0.9 ± 1.9b	8.6 ± 5.3a	8.4 ± 1.9a	3.0 ± 4.6b
0.2	6.1 ± 3.7a	3.9 ± 2.0ab	0 c	8.9 ± 3.6a	7.6 ± 1.6a	0 c
0.5	1.6 ± 1.9b	0 c	-[a]	5.5 ± 0.6b	0 c	-[a]

[a] All flies died one week after treatment.

Means within the column followed by the same letter are not significantly different ($p = 0.05$, Tukey's HSD test).

Table 3-11. Mean fecundity of *A. suspensa* treated as 10-d-old flies with various concentrations of sodium tetraborate by feeding different periods.

	Eggs/female/day (Mean ± SD)					
	Day 1-7 after treatment			Day 1-20 after treatment		
Conc. (%)	24 hr treatment	48 hr treatment	168 hr treatment	24 hr treatment	48 hr treatment	168 hr treatment
0	15.0 ± 4.5ab	10.7 ± 3.3a	15.0 ± 5.2a	14.8 ± 2.9a	11.6 ± 3.1a	14.6 ± 2.6a
0.02	18.9 ± 6.2a	13.3 ± 1.2a	15.2 ± 4.2a	15.7 ± 4.4a	12.0 ± 1.4a	14.9 ± 2.6a
0.05	9.0 ± 2.2bc	15.1 ± 4.1a	12.6 ± 2.3ab	12.8 ± 1.6a	14.8 ± 4.9a	13.9 ± 2.5ab
0.1	14.3 ± 2.2ab	14.8 ± 2.2a	6.9 ± 3.7bc	14.8 ± 2.3a	13.3 ± 1.2a	10.1 ± 4.0b
0.2	12.5 ± 3.3ab	10.6 ± 4.9a	3.6 ± 2.2c	13.2 ± 3.3a	12.6 ± 2.7a	5.0 ± 3.0c
0.5	5.1 ± 2.5bc	3.0 ± 1.8b	0.8 ± 0.6c	9.4 ± 3.3b	5.9 ± 3.6b	0.8 ± 0.6c [a]

[a] All flies died one week after treatment.

Means within the column followed by the same letter are not significantly different (p = 0.05, Tukey's HSD).

Table 3-12. Mean fertility of *A. suspensa* treated with various concentrations of sodium tetraborate by feeding different periods.

	Egg hatch (%) (Mean ± SD)					
	Newly emerged fly treatment			10-d-old fly treatment		
Conc. (%)	24 hr treatment	48 hr treatment	168 hr treatment	24 hr treatment	48 hr treatment	168 hr treatment
0	80.5 ± 27.2 a	79.7 ± 28.0a	79.2 ± 26.8a	83.7 ± 15.3a	86.9 ± 14.8a	82.5 ± 15.0a
0.02	85.2 ± 20.2a	82.2 ± 27.0a	70.8 ± 23.3a	87.7 ± 15.4a	85.6 ± 15.8a	74.6 ± 21.1a
0.05	86.0 ± 20.6a	82.6 ± 22.9a	75.3 ± 25.5a	83.7 ± 14.9a	80.4 ± 15.4a	86.2 ± 13.6a
0.1	86.4 ± 19.5a	72.2 ± 34.5a	23.8 ± 12.5b	75.4 ± 22.8a	83.6 ± 14.4a	75.5 ± 20.1a
0.2	77.6 ± 28.3a	75.4 ± 33.1a	-[a]	81.9 ± 15.7a	69.7 ± 26.6a	42.6 ± 27.8b
0.5	55.0 ± 26.3b	-[a]	-[b]	65.4 ± 27.2b	55.0 ± 32.9b	10.6 ± 14.9c[a]

[a] No eggs were oviposited by survivors.

[b] All flies died one week after treatment.

Means within the column followed by the same letter are not significantly different ($p = 0.05$, Tukey's HSD test).

Table 3-13. LT_{50}s [a] of sodium tetraborate to *A. suspensa* treated as newly emerged adult by feeding one week on choice or no choice food.

	Male		Female	
Conc. (%)	LT_{50} (95% CI)	Slope ± SE	LT_{50} (95% CI)	Slope ± SE
No choice				
0.2	5.29 [b]	8.40 ± 81.9	6.04 [b]	9.92 ± 24.2
0.5	3.72 (3.42 – 4.00)	9.55 ± 2.14	4.31 (4.03 – 4.57)	13.4 ± 4.87
1.0	2.53 (2.33 – 2.74)	6.61 ± 2.19	2.98 (2.73 –3.21)	11.9 ± 4.42
Choice				
0.2	>20	--	>20	--
0.5	5.77 [b]	4.64 ± 17.3	6.03 [b]	7.12 ± 29.4
1.0	4.41 (4.10 – 4.73)	10.6 ± 2.97	4.79 (3.09 –8.79)	11.9 ± 4.42

[a] LT_{50} was expressed as day.

[b] Poor fit of probit model prevented estimation of 95% CI.

Table 3-14. LT_{50}s [a] of sodium tetraborate to *A. suspensa* treated as 10-d-old adult by feeding one week on choice or no choice food.

	Male		Female	
Conc. (%)	LT_{50} (95% CI)	Slope ± SE	LT_{50} (95% CI)	Slope ± SE
No choice				
0.2	4.72 (4.33 – 5.10)	7.06 ± 0.85	5.38 [b]	4.57 ± 5.25
0.5	2.67 (2.44 – 2.89)	12.3 ± 4.85	3.01 [b]	6.12 ± 5.48
1.0	1.96 (1.67 – 2.22)	5.96 ± 0.93	2.80 (2.55 –3.05)	9.72 ± 2.49
Choice				
0.2	>20	–	>20	–
0.5	4.62 (4.31 – 4.92)	10.0 ± 2.22	5.87 (5.29 – 6.50)	4.36 ± 0.30
1.0	2.42 (2.10 – 2.71)	5.97 ± 0.86	3.46 (3.12 –3.78)	7.17 ± 1.10

[a] LT_{50} was expressed as day.

[b] Poor fit of probit model prevented estimation of 95% CI.

Table 3-15. Mean fecundity of *A. suspensa* treated as newly emerged flies by feeding one week with various concentrations of sodium tetraborate.

		Eggs/female/day (Mean ± SD)			
		Day 7-13 after treatment		Day 7-20 after treatment	
Conc. (%)	N	Choice	No choice	Choice	No choice
0	5	19.8 ± 5.0 a	19.8 ± 5.0 a	18.9 ± 2.9 a	18.9 ± 2.9 a
0.1	5	15.3 ± 7.3 a	4.0 ± 2.1 b	13.6 ± 5.2 ab	7.3 ± 1.8 b
0.2	5	10.5 ± 6.2 ab	- [a]	12.0 ± 5.3 ab	- [b]
0.5	5	- [a]	- [b]	- [a]	- [b]
1.0	5	- [b]	- [b]	- [b]	- [b]

[a] No eggs were oviposited by survivors.

[b] All flies died 7 d after treatment.

Means in each oviposition period followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

Table 3-16. Mean fecundity of *A. suspensa* treated as 10-d-old flies by feeding one week with various concentrations of sodium tetraborate.

		Eggs/female/day (Mean ± SD)			
		Day 1-7 after treatment		Day 1-20 after treatment	
Conc. (%)	N	Choice	No choice	Choice	No choice
0	5	12.1 ± 4.8 a	12.1 ± 4.8 a	14.9 ± 2.2 a	14.9 ± 2.2 a
0.1	5	12.8 ± 4.4 a	5.6 ± 2.1 bc	11.1 ± 3.4 ab	8.7 ± 3.2 b
0.2	5	10.1 ± 3.4 ab	2.7 ± 2.1 c	10.2 ± 4.3 ab	2.4 ± 2.2 c
0.5	5	2.4 ± 2.0 c	1.2 ± 0.7 c	1.5 ± 0.9 c	1.2 ± 0.7 c [a]
1.0	5	1.9 ± 1.1 c	0.7 ± 0.9 c	1.9 ± 1.1 c [a]	0.7 ± 0.9 c [a]

[a] All flies died 7 d after treatment

Means in each oviposition period followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

Table 3-17. Mean egg hatch of *A. suspensa* treated as newly emerged flies by feeding for one week with sodium tetraborate.

		Egg hatch (%)(Mean ± SD			
		Day 7-13 after treatment		Day 7-20 after treatment	
Conc. (%)	N	Choice	Non-choice	Choice	Non-choice
0	5	83.9 ± 13.6 a	83.9 ± 13.6 a	82.4 ± 13.2 a	82.4 ± 13.2 a
0.1	5	71.1 ± 25.1 a	31.0 ± 21.2 b	75.9 ± 22.1 a	27.2 ± 21.2 b
0.2	5	55.8 ± 26.9 b	- [a]	61.1 ± 26.0 b	- [a]
0.5	5	- [a]	- [a]	- [a]	- [ab]
1.0	5	- [a]	- [a]	- [ab]	- [ab]

[a] No eggs were oviposited by survivors.

[b] All flies died 7 d after treatment

Means in each oviposition period followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

Table 3-18. Mean egg hatch of *A. suspensa* treated as 10-d-old flies by feeding for one week with sodium tetraborate.

		Egg hatch (%)(Mean ± SD)			
		Day 1-7 after treatment		Day 1-20 after treatment	
Conc. (%)	N	Choice	No choice	Choice	No choice
0	5	86.9 ± 10.8 a	86.9 ± 10.8 a	86.4 ± 13.6 a	86.4 ± 13.6 a
0.1	5	80.9 ± 20.3 ab	60.7 ± 29.6 ab	79.0 ± 17.0 ab	55.5 ± 29.4 ab
0.2	5	64.1 ± 31.0 abc	35.4 ± 32.6 bc	69.9 ± 23.8 abc	35.4 ± 32.6 bc
0.5	5	40.7 ± 39.9 c	23.8 ± 11.7 bc	40.7 ± 39.9 c	23.8 ± 11.7 bc [a]
1.0	5	45.0 ± 29.2 c	11.2 ± 15.9 c	45.0 ± 29.2 c [a]	11.2 ± 15.9 c [a]

[a] All flies died 7 d after treatment.

Means in each oviposition period followed by the same letter are not significantly different
(P = 0.05, Tukey's HSD test).

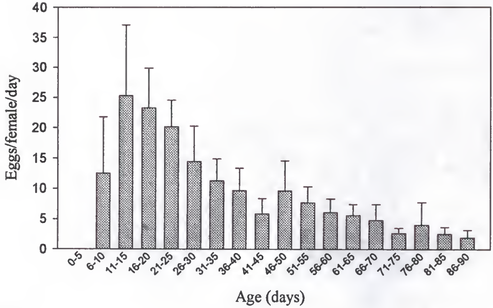


Figure 3-1. Oviposition pattern of *A. suspensa.*

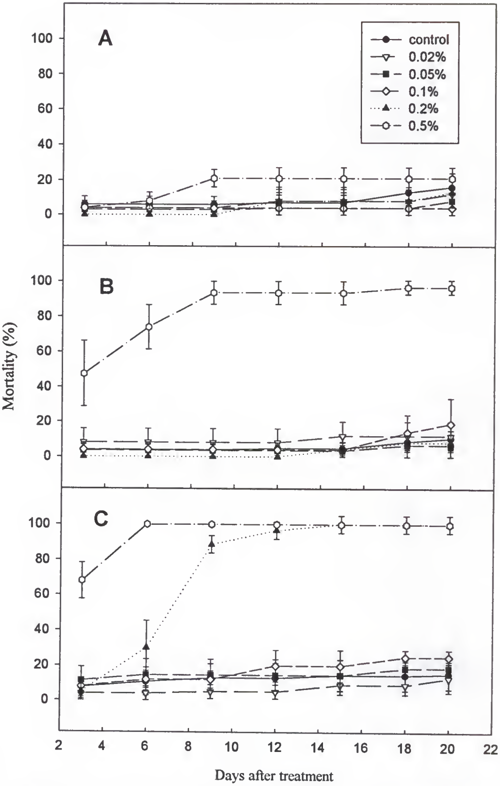


Figure 3-2. Mortality of male *A. suspensa* treated as newly emerged adults with sodium tetraborate by feeding 24hr (A), 48hr (B), and 168hr (C).

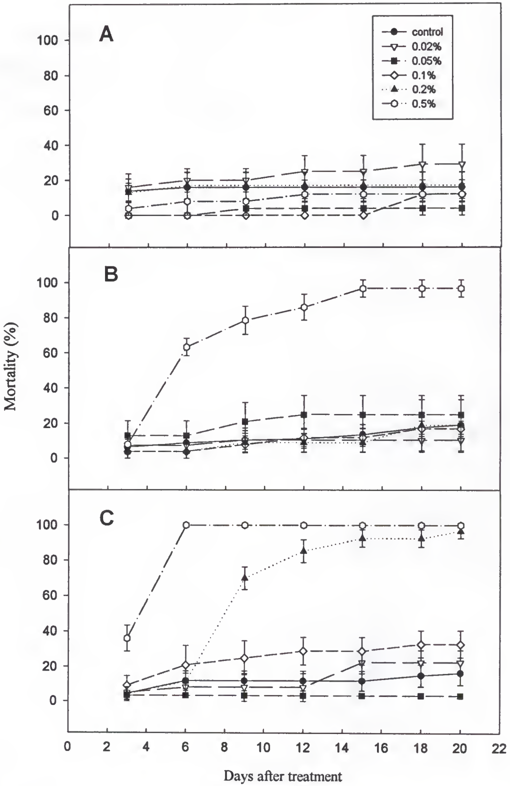


Figure 3-3. Mortality of female *A. suspensa* treated as newly emerged adults with sodium tetraborate by feeding 24hr (A), 48hr (B), and 168hr (C).

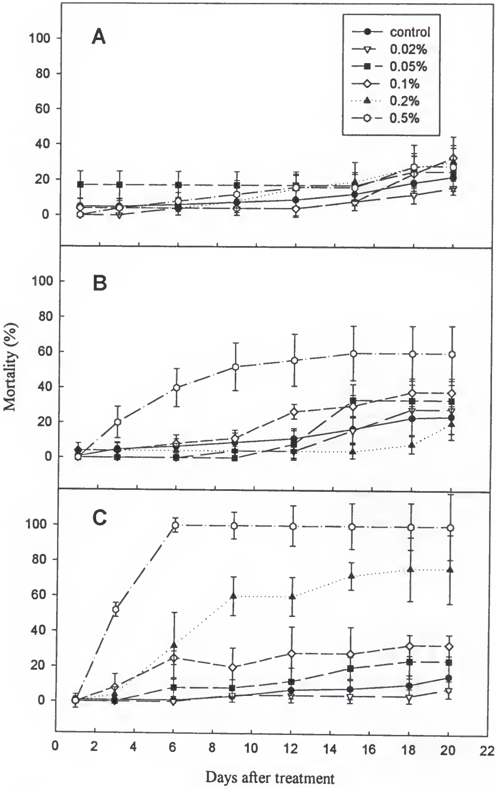


Figure 3-4. Mortality of male *A. suspensa* treated as 10-d-old adults with sodium tetraborate by feeding 24hr (A), 48hr (B), and 168hr (C).

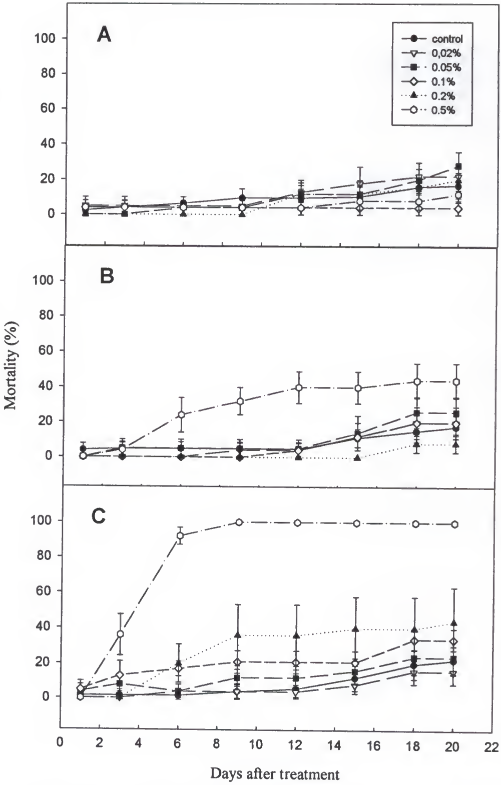


Figure 3-5. Mortality of female *A. suspensa* treated as 10-d-old adults with sodium tetraborate by feeding 24hr (A), 48hr (B), and 168hr (C).

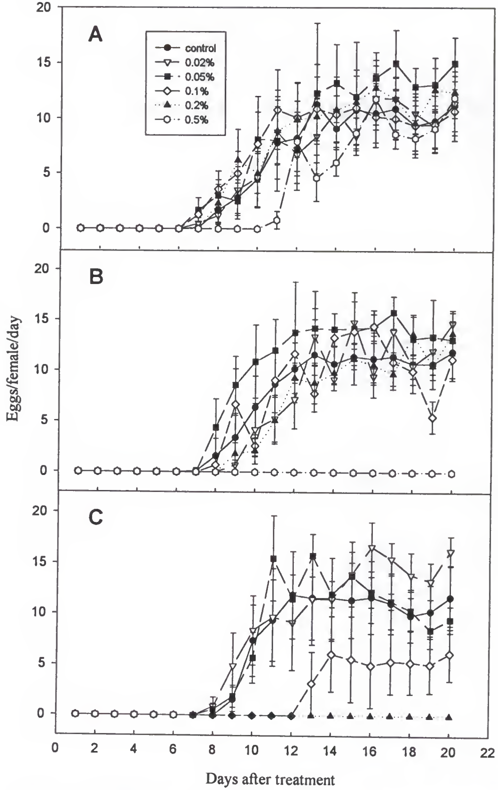


Figure 3-6. Mean fecundity of female *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 24hr (A), 48hr (B), or 168hr(C).

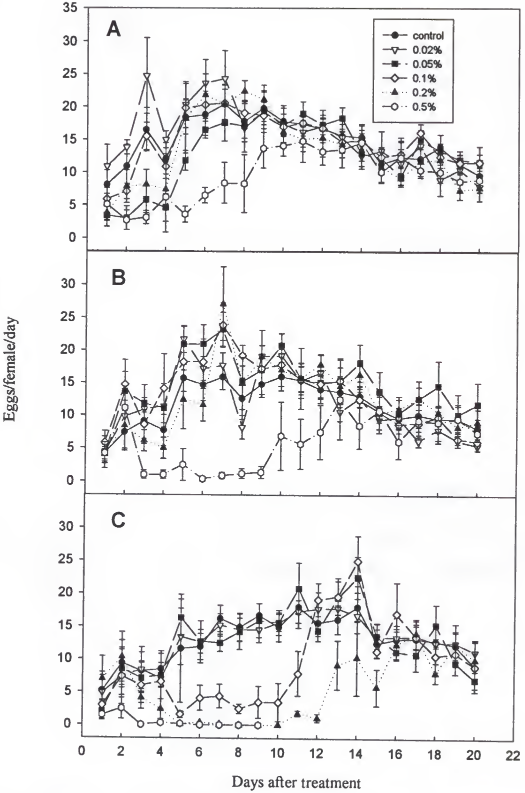


Figure 3-7. Mean fecundity of female *A. susspensa* treated as 10-d-old adults with sodium tetraborate by feeding 24 hr (A), 48 hr (B), or 168hr (C).

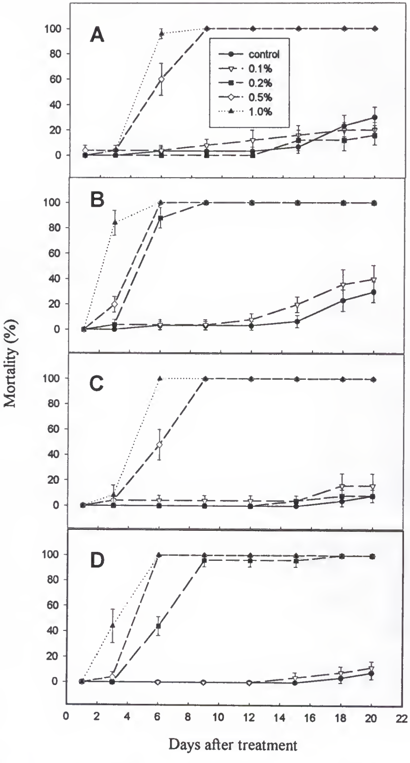


Figure 3-8. Mortality of *A. suspensa* treated as newly emerged adult with sodium tetraborate by feeding 168hr on choice food (A&C) or no choice food (B&D), A: Male, B: Male, C: Female, D: Female.

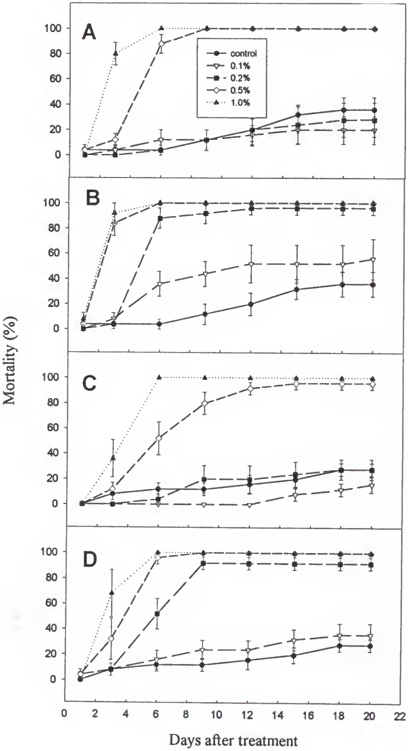


Figure 3-9. Mortality of *A. suspensa* treated as 10-d-old adults with sodium tetraborate by feeding 168hr on choice food (A&C) or no choice food (B&C), A: Males, B: Males, C: Females, D: Females.

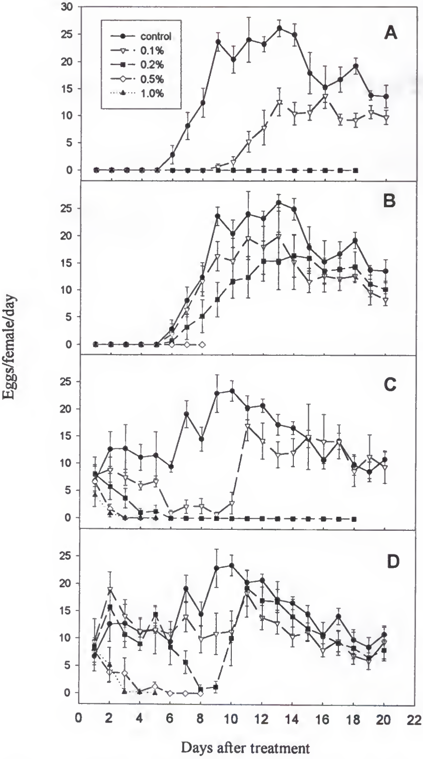


Figure 3-10. Mean fecundity of *A. suspensa* treated as newly emerged adults (A&B) or 10-d-old adult (C&D) by feeding 168 hr on choice or no choice food, A: No choice; B: Choice; C: No choice; D: Choice.

CHAPTER 4
MORPHOLOGICAL EFFECTS OF SODIUM TETRABORATE AND IMIDACLOPRID ON OVARIAN DEVELOPMENT OF *ANASTREPHA SUSPENSA*

Introduction

Anastrepha suspensa (Loew) (Caribbean fruit fly) is an economically important insect of tropical and subtropical fruit in Florida, and infests 80 species of tropical and subtropical fruits in 23 plant families (Swanson & Baranowski, 1972).

Ovarian development in *Drosophila melanogaster* has been divided into a series of 14 stages (King, 1970), beginning with the terminal 16-cell cluster in the germanium and ending with the mature egg in the ovariole; vitellogenesis occurs during stages 8 through 12. These characters outlined for *Drosophila melanogaster* have been adopted for many other Dipteran families (Buning, 1997). In the house fly, *Musca domestica,* 10 stages of oogenesis have been described during ovarian development, vitellogenin accumulation start at stage 4 (Adams, 1974). During ovarian development, vitellogenin was synthesized by the fat body and follicle cells of the ovary and then up taken by the developing oocyte. When the ovary approached maturity, nurse cells supplied all synthesized RNA and cytoplasm to the oocyte and then degenerated; follicle cells disappeared after the vitellin membrane and chorion, egg shell, were formed.

The morphological effects of chemicals on ovarian development have been investigated in some insects. Hsu et al. (1989) reported that terminal follicles in the mature ovaries of Mediterranean fruit flies, *Ceratitis capitata*, lacked well-defined nurse cells and follicular epithelia when newly emerged flies were treated orally with 5-ethoxy-6-(4-methoxyphenyl) methyl-1, 3-benzodioxole. In the cat flea, *Ctenocephalides felis*, treated with pyriproxyfen, the development of the follicular epithelium, nuclei, and yolk sphere in the oocytes of the 7-d-old flea were destroyed (Meola et al., 1996).

Sodium tetraborate has been used as a wood preservative and to control bacteria, algae, fungi, and insects (Lloyd, 1998). In the screw-worm fly, *Cochliomyia hominivorax*, (Settepani et al., 1969) and the house fly, *M. domestica* (Borkovec et al., 1969), concentrations above 1% produced some mortality and stopped oviposition. Work in our laboratory (Nigg & Simpson, 1997) showed that oviposition of *A. suspensa* was delayed up to 7 d after a single oral treatment with sodium tetraborate.

Imidacloprid is a chloronicotinyl insecticide found to be effective against many economically important insect pests such as aphids, whiteflies, and beetles (Palumbo et al., 1996; Boiuteau et al., 1997).

The objective of this study was to examine the morphological changes of the ovaries in *A. suspensa* treated with sodium tetraborate and imidacloprid.

Materials and Methods

Insects

A. suspensa were obtained as 9-d-old pupae from the Florida Department of Agricultural and Consumer Services, Division of Plant Industry, Gainesville, FL. Flies which emerged over 4 hr were placed in 950 cm^3 plastic containers with 10 female and 10 male flies in each. One group was treated immediately with 0.02, 0.05, 0.1, 0.2, and 0.5% sodium tetraborate or 0.05, 0.1, 0.2, 0.5, and 1.0 mg/l imidacloprid. A second group was treated at different ages by feeding 0.5% sodium tetraborate or 1.0 mg/l imidacloprid for 24 hr. The food with sodium tetraborate or imidacloprid was removed after 24 hr and changed to normal food. On day 1 after emergence, one egg laying square was placed on the top of each container to monitor oviposition (Nigg et al., 1994). Control flies were treated in exactly the same manner except that no pesticide(s) was added to their food. All flies were held in the laboratory at 25 to 28 °C and 50 to 70% RH, with a photoperiod of L:D 12:12.

Chemicals

Nonenyl succinic anhydride (NSA), vinyl cyclohexene dioxide (ERL4206), epoxy resin (DER736), and 2-dimethylamino ethanol were bought from Ted Pella, Inc. (Redding, CA). Methylene blue-azure A, basic fuchsin, hexamethyldisilazane (HMDS), and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Specimen Preparation for SEM

Female *A. suspensa* were dissected in 3% glutaraldehyde in 0.1 M potassium phosphate (KPO_4) buffer (pH 7.2) at day 7 after emergence, except for the time course experiment in which female flies were dissected daily after emergence up to day 7. Ovary preparation prior to coating followed the method described by Nation (1983). After dissection, ovaries were placed in buffered glutaraldehyde for 5 min, transferred into 0.1 M KPO_4 buffer and then dehydrated through ethanol solutions of 70%, 85%, 95%, and 100% (5 min in each). The ovaries were immersed then in HMDS for 5 min and dried in a desiccator for 24 hr. The ovaries were mounted on stainless steel stubs with double stick tabs, and coated immediately with gold/palladium using a sputter coater (Ladd Research Industries, Burlington, VE) for 90 sec.

Specimen Preparation for Light Microscopy

On day 7 after emergence, female *A. suspensa* from controls and treatments were dissected in 3% glutaraldehyde in 0.1 M KPO_4 buffer (pH 7.2). Ovaries were dehydrated with ethanol and HMDS as previously described. After drying in a desiccator overnight, ovaries were infiltrated with 5:5, 7:3, and 10:0 of Spurr's resin solution: acetone for 8 hr, respectively. Ovaries were embedded by placing them in molds, covering with fresh resin, and placing in a 70 °C oven for 24 hr (Spurr, 1969). Embedded ovaries were sectioned to 1-2 μm on an ultramicrotome (Huxley; LKB Instruments, Inc., Rockville, MD). Sections were affixed to slides by floating on a drop of distilled water and heating on a hot plate at 70 °C until the water evaporated. Sections were stained then in methylene blue-azure A solution (0.13 mg methylene blue and 0.02 mg azure A were dissolved in 10 ml glycerol, 10 ml methanol and 30 ml phosphate buffer, pH 6.9, and diluted with 50 ml distilled water) at 70 °C for 1-2 min, and then in fuchsin solution (0.1 mg basic fuchsin was dissolved in 10 ml of 50% methanol and diluted 20 times with distilled water) at room temperature for 30 sec (Schneider, 1981). Section slides were rinsed with distilled water after each staining. Finally, sections on slides were dried on a 70°C hot plate, wetted with 50 µl of immersion oil, and covered with a coverslip. The coverslip was sealed with clean fingernail polish.

Morphological Examination

Coated ovaries were viewed and photographed with a SEM S530 with an integral camera (Hitachi, Ltd., Tokyo) using Polaroid film. Stained ovarian sections were viewed with a Vanox T light microscope (Olympus Optical Co., Ltd., Tokyo) and photographed using ASA 100 Kodak film, and a green filter. Negatives were printed using multigrade Kodak resin coated paper.

In a separate 3 replicate experiment, 8 newly-emerged females and 8 newly-emerged males were caged in 950 cm^3 plastic containers and fed different doses of sodium tetraborate or imidacloprid as described above. A separate 3 replicate set of flies was fed 0.5% sodium tetraborate or 1.0 mg/l imidacloprid for 24 hr at ages 0, 1, 3, and 5 d. On day 7, the flies were stored in a –70 °C freezer. The ovaries were removed intact and measured with a light microscope for length, apex to apex, and width at their widest point. Data were analyzed by the general linear model procedure (SAS Institute, 1989). Significant differences among control and treatments (P = 0.05) were tested with Tukey's HSD test (SAS Institute, 1989). The number of samples (n) in the tables depended upon fly deaths because only survivors were dissected and the number of ovaries undamaged in dissection. The number of flies dissected may be obtained generally by dividing n by 2.

Results and Discussion

Normal ovarian development of *A. suspensa* is shown in Table 4-1 and Figure 4-1. Unlike *D. melanogaster* (King, 1970) and *M. domestica* (Adams, 1974) which require 2-3 d to develop their mature egg from eclosion at 25-27 °C. *A. suspensa* needed 6-7 d to produce a mature egg. The increase in ovary size between 1-d-old and 7-d-old flies was about 50 times; ovarian development in the 2-3 d prior to oviposition was faster than in the first 3 d after eclosion.

The effects of sodium tetraborate and imidacloprid on ovarian development of *A. suspensa* are shown in Tables 4-2 - 4-5 and Figs. 4-2 - 4-5. Table 4-2 and Fig. 4-2 show that ovarian development of *A. suspensa* was inhibited to different degrees when newly emerged flies were treated with different concentrations of sodium tetraborate; higher concentrations caused greater size reduction. When different age flies were treated with 0.5% sodium tetraborate, ovarian development of *A. suspensa* from all treatments was reduced significantly (Table 4-3 and Fig. 4-3). Ovarian development of 7-d-old flies from a 0.5% treatment was reduced to that of 3- to 4-d-old untreated fly. This result is similar to that reported by Walder & Calkins (1992) for *A. suspensa* pupae exposed to gamma radiation.

For flies treated with imidacloprid, whether treated as newly emerged adults with different concentrations or treated with 1.0 mg/l at different ages, no difference in ovary dimensions were observed among treatment and control ovaries (Tables 4-4 and 4-5). Figs. 4-4 and 4-5 show that when flies were treated with 1.0 mg/l imidacloprid, ovarian development was reduced to a certain degree, since ovaries with less than normal yolk contracted after being dehydrated. These reductions were less than that of flies treated with 0.5% sodium tetraborate (Figs. 4-2 and 4-3).

The differences in ovarian ultrastructure in *A. suspensa* among control and treatments are shown in Fig. 4-6. For normal *A. suspensa*, yolk accumulation began around day 3 after eclosion (Handler & Shirk, 1988; Handler, 1997). The stage of oogenesis at this time is similar to stage 8 in *D. melanogaster* (King, 1970), and stage 4 in *M. domestica* (Adams, 1974). At these stages, vitellogenesis start to occur. Figure 4-6 shows the effects on the oocyte in flies treated with 1.0 mg/l imidacloprid or 0.5% sodium tetraborate. For 1.0 mg/l imidacloprid treated flies, the follicular epithelium of the oocyte did not grow well. In 0.5% sodium tetraborate treated flies, yolk accumulation in the oocyte was greatly reduced, and some oocytes lacked well-defined nurse cells. These results were similar to the results on Mediterranean fruit fly treated with a benzodioxole (Hsu et al., 1989) and cat flea treated with pyripoxyfen (Meola et al., 1996). It appears from these data that the cessation of oviposition after sodium tetraborate ingestion (Nigg & Simpson, 1997) is due to inhibition of the production or the uptake of the yolk protein, or inhibition of both processes. Less than 1.0 mg/l imidacloprid had less or no effect on ovarian development of *A. suspensa* after 24 hr treatment.

Same as the results reported in the last chapter, yolk protein accumulation and /or ovarian development of *A. suspensa* were not affected by ≤0.2% sodium tetraborate, but were reduced greatly by 0.5% concentration when flies were treated by feeding 24hr regardless of fly age. The ≤1.0mg/l imidacloprid treatment had no effect on ovarian development of *A. suspensa* after 24hr treatment regardless of fly age.

Table 4-1. Time course of ovary development of *A. suspensa*

Age (day)	N	Length (mm)	Width (mm)
0	24	0.51 ± 0.13 c	0.41 ± 0.09 e
1	26	0.54 ± 0.14 c	0.39 ± 0.10 e
2	24	0.60 ± 0.12 c	0.48 ± 0.08 de
3	24	0.69 ± 0.20 c	0.55 ± 0.10 cd
4	24	0.89 ± 0.17 b	0.65 ± 0.11 c
5	26	1.03 ± 0.22 b	0.79 ± 0.16 b
6	24	1.44 ± 0.28 a	1.02 ± 0.14 a
7	24	1.46 ± 0.38 a	0.97 ± 0.22 a

Means in each column followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test).

Table 4-2. Ovary dimensions of 7-d-old *A. suspensa* treated as newly emerged adult with different concentrations of sodium tetraborate by feeding 24 hr.

Conc (%)	N	Length (mm)	Width (mm)
0	39	1.58 ± 0.29 a	1.13 ± 0.25 a
0.02	41	1.57 ± 0.46 a	1.14 ± 0.25 a
0.05	36	1.43 ± 0.38 a	1.04 ± 0.25 ab
0.1	37	1.57 ± 0.39 a	1.12 ± 0.27 ab
0.2	40	1.35 ± 0.31 a	0.98 ± 0.22 b
0.5	40	0.80 ± 0.24 b	0.59 ± 0.18 c

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

Table 4-3. Ovary dimensions of 7-d-old *A. suspensa* treated at different ages with 0.5% of sodium tetraborate by feeding 24 hr.

Treatment age (day)	N	Length (mm)	Width (mm)
0	40	0.80 ± 0.24 d	0.59 ± 0.18 c
1	41	0.92 ± 0.24 cd	0.70 ± 0.18 c
3	36	1.05 ± 0.22 bc	0.86 ± 0.13 b
5	41	1.15 ± 0.22 b	0.92 ± 0.17 b
Control	39	1.58 ± 0.29 a	1.14 ± 0.25 a

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

Table 4-4. Ovary dimensions of 7-d-old *A. suspensa* treated as newly emerged adult with different concentrations of imidacloprid by feeding 24 hr.

Conc (mg/l)	N	Length (mm)	Width (mm)
0	36	1.50 ± 0.35 ab	1.12 ± 0.27 abc
0.05	35	1.36 ± 0.27 b	0.93 ± 0.20 c
0.1	36	1.48 ± 0.38 ab	1.06 ± 0.24 bc
0.2	36	1.70 ± 0.47 a	1.27 ± 0.31 a
0.5	38	1.48 ± 0.37 ab	1.16 ± 0.26 ab
1.0	36	1.28 ± 0.40 b	1.01 ± 0.28 bc

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

Table 4-5. Ovary dimensions of 7-d-old *A. suspensa* treated at different ages with 1.0 mg/l imidacloprid by feeding 24 hr.

Treatment age (day)	N	Length (mm)	Width (mm)
0	36	1.28 ± 0.40 ab	1.01 ± 0.28 ab
1	36	1.24 ± 0.35 b	0.97 ± 0.30 b
3	34	1.32 ± 0.31 ab	1.05 ± 0.28 ab
5	34	1.52 ± 0.26 a	1.20 ± 0.23 a
Control	36	1.33 ± 0.31 ab	1.01 ± 0.25 ab

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

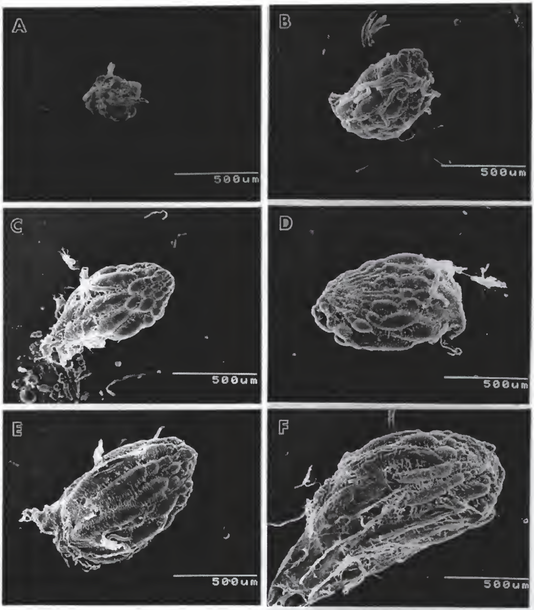


Figure 4-1. Time course of ovarian development in *A. suspensa*. A: 1-d-old; B: 3-d-old; C: 4-d-old; D: 5-d-old; E: 6-d-old; F: 7-d-old.

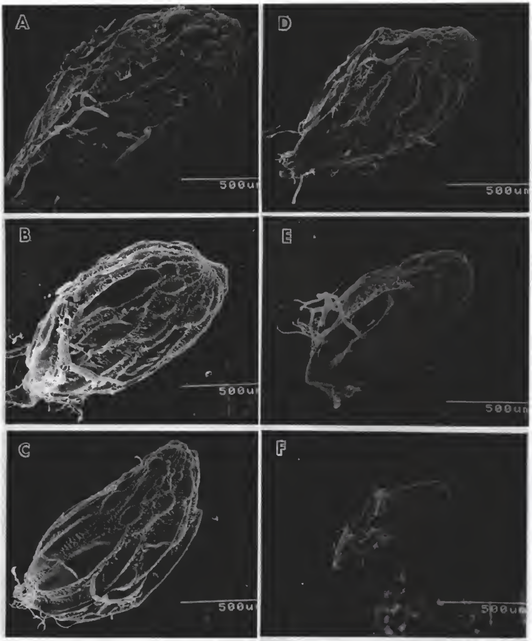


Figure 4-2. Ovarian development in 7-d-old *A. suspensa* treated as newly emerged adults with sodium tetraborate. A: control; B: 0.02%; C: 0.05%; D: 0.1%; E: 0.2%; F: 0.5%.

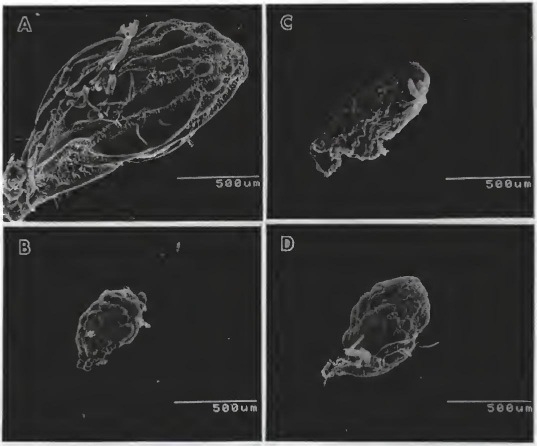


Figure 4-3. Ovarian development of 7-d-old *A. suspensa* treated as adults at different ages with 0.5% sodium tetraborate. A: control, B: day 1, C: day 3, D: day 5.

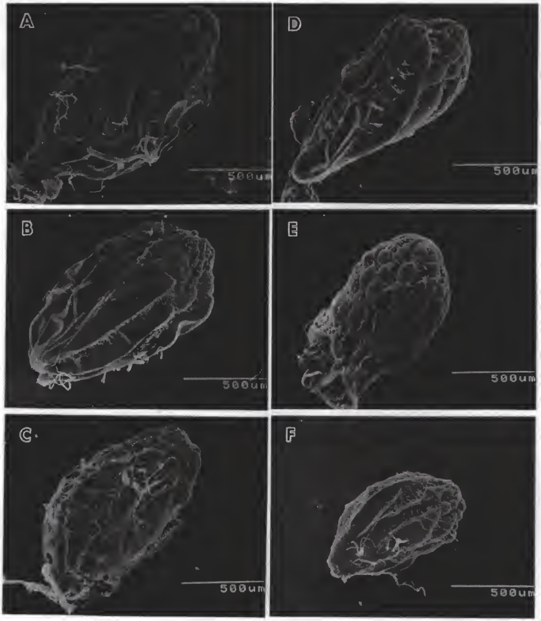


Figure 4- 4. Ovarian development in 7-d-old *A. suspensa* treated as newly emerged adults with imidacloprid. A: control, B: 0.05mg/l, C: 0.1mg/l, D: 0.2mg/l, E: 0.5mg/l, F: 1.0mg/l.

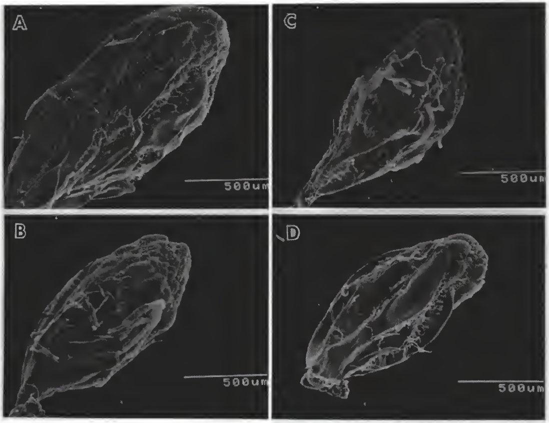


Figure 4- 5. Ovarian development in 7-d-old *A. suspensa* treated as adults at different ages with 1.0 mg/l imidacloprid. A: control, B: day 1, C: day 3, D: day 5.

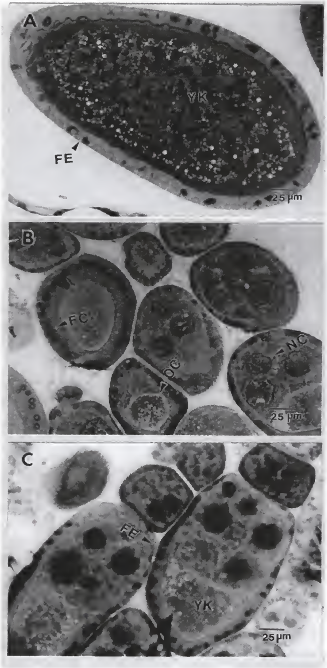


Figure 6- 6. Light microscopy of oocytes in 7-d-old *A. suspensa* treated as newly emerged adults with sodium tetraborate (0.5%) (B) or imidacloprid (1.0mg/l) (C), (A): control; FC: follicle cell, FE: follicular epithelium, NC: nurse cell, OC: oocyte, YK: yolk protein.

CHAPTER 5
EFFECT OF SODIUM TETRABORATE AND IMIDACLOPRID ON PROTEINASE ACTIVITIES OF FEMALE *ANASTREPHA SUSPENSA*

Introduction

Insects require an adequate quality and quantity of protein for normal development and reproduction (Ferro & Zucoloto, 1990). The digestive system in insects has adapted to specific food sources by synthesizing specific classes of digestive enzymes (Baker, 1981). In the midgut, endoproteinases including serine and cysteine proteinases, and exoproteinases including aminopeptidase and carboxypeptidases A and B, have been studied in various insects (Applebaum, 1985; Houseman et al., 1991; Christeller et al., 1992). Endoproteinases are defined as proteinases cleaving internal peptide bonds; exoproteinases remove terminal amino acids from either the carboxyl or the amino ends (Applebaum, 1985; Barrett, 1994). Serine proteinases have been demonstrated in many different insect orders, such as Orthoptera (Knecht et al., 1974), Coleoptera (Purcell et al., 1992; Houseman & Thie, 1993), Lepidoptera (Christeller et al., 1992; Johnston et al., 1995, Valaitis, 1995), and Diptera (Sharma et al., 1984; Moffatt & Lehane, 1990). These serine proteinases were characterized as trypsin- and chymotrypsin-like enzymes by their alkaline pH optima and responsiveness to specific substrates and inhibitors. In some Coleoptera and Hemiptera, cysteine proteinases were found to play a major digestive role

(Murdock et al., 1987, Houseman & Thie, 1993). Exoproteinases, such as aminopeptidase and carboxypeptidase A and B, have been reported in some hematophagous insects, such as the blood-sucking bug, *Rhodnius prolixus* (Houseman & Downe, 1981, 1983), and *Stomoxys calcitrans* (Schneider et al., 1987), and some phytophagous insects, such as corn earworm, *Heliothis zea* (Lenz et al., 1991), and the stalk corn borer, *Sesamia conagriaides* Lef. (Ortego et al., 1996).

Insect egg proteinases have been investigated only in a few species (Greenberg & Paretsky, 1955; Shulov et al., 1957; Kageyama et al., 1981; Medina et al., 1988; Ikeda et al., 1990; Ribolla et al., 1993). Insect egg proteinases have a pH optimum in the acidic pH region, activities were inhibited by cysteine specific inhibitors and/or activated by thiol compounds, and therefore these proteinases were recognized as cysteine proteinases (Houseman & Thie, 1993). Based on substrate specificity and amino acid sequence, they were characterized further as cathepsin B- (Medina et al., 1988) and Cathepsin L-like cysteine proteinases (Kageyama & Takahashi, 1990). Besides cysteine proteinase, trypsin-like proteinase has also been found in the eggs of *Locusta migratoria* (Shulov et al., 1957) and *Bombyx mori* (Ikeda et al., 1990). These proteinases apparently catabolize yolk proteins for utilization during embryogenesis (Kagerama & Takahash, 1990).

A. suspensa is a polyphagous herbivore with a broad spectrum of tropical and subtropical fruit hosts, including common guava, *Psidium guajava* L., surinam cherry, *Eugenia uniflora* L. and calamondin, *Citrus mitis* Blanco (Swanson & Baranowski, 1972). Although many studies related to the biology and ecology of *A. suspensa* have been conducted (Nation, 1972, 1990, Mazomenos et al., 1977, Burk, 1983, Webb et al., 1984, Sivinski & Heath, 1988, Nigg et al., 1994), no data have been generated on midgut digestive proteinases or on egg proteinases in *A. suspensa*. The purpose of this study was to determine the inhibition of *A. suspensa* proteinases as a possible mechanism for the egg laying effect of sodium tetraborate compound and imidacloprid.

Materials and Methods

Chemicals and Equipment

Sodium tetraborate was purchased from Fisher Scientific, Fair Lawn, NJ and imidacloprid from Miles Inc. (Kansas City, MO). All enzyme substrates and inhibitors were obtained from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric measurements were made using a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Columbia, MD).

Insect Treatment

Pupae (9 d old) of *A. suspensa* were shipped overnight from the Florida Department of Agricultural and Consumer Services, Division of Plant Industry, Gainesville, FL. Flies which emerged over 4 hr were placed in 30.5 cm^3 cages (Bio Quip, Inc., Gardero CA), approximately 1500 flies per cage, and were treated immediately with 0.2% and 0.5% sodium tetraborate and 0.5 mg/l and 1.0 mg/l imidacloprid by feeding for 24 hr according to Nigg et al. (1994). These concentrations were chosen from preliminary experiments in which less than 10% of flies were killed at these concentrations. The food with pesticides was removed after 24 hr and changed to normal food (yeast hydrolysate enzymatic:sugar 1:3 as a patty and a 1% agar paddy for water; Nigg et al., 1994). Control flies were treated in exactly the same manner except that no pesticide(s) was added to their food. There were 3 replicate cages for each treatment over 3-4 weeks. All flies were held in the laboratory at 25 to 28 °C and 50 to 70% RH, with a photoperiod of L:D 12:12. For the time course experiment, 3 cages of about 1500 flies were prepared from the same pupae set. Flies were collected daily from day 0 to day 10 after emergence. On each sampling day, flies collected from each cage were combined as 1 sample, 3 samples total.

Proteinase Preparation

For midgut proteinase preparation, the control and treatment samples were taken 24, 48, 96, and 144 hr after treatment. The flies were dissected in 0.09% saline solution. Midguts were removed, externally rinsed with glass distilled, deionized water (DDW), and were stored in 0.1 M pH 8.0 sodium phosphate buffer; 10 midguts/200 μl buffer at -40 °C until needed. For ovary proteinase preparation, female flies were collected from control and treatment cages on day 7 after emergence and dissected in saline solution. The ovaries were removed and stored the same as midgut samples.

Midguts and ovaries were manually homogenized with a plastic microgrinder in 0.1 M sodium phosphate buffer (pH 8.0), centrifuged at 10,000 × g for 10 min, and the supernatants used for proteinase activity and protein quantification. Samples were always kept in ice or at 4 °C during preparation and were used immediately after preparation.

For the determination of pH optima, the midgut extract from 4- to 6-d-old female flies and the ovary extract from 7-d-old flies were used as proteinase sources. Inhibition studies were conducted by addition of specific inhibitors to midgut or ovary extracts and incubation for 20 min at 30 °C before substrates were added. Preliminary studies used 5, 10, 15, and 20 min for inhibitor incubation. There was no difference in inhibition comparing 15 and 20 min at the inhibitor concentrations used. Results are expressed as a percentage of activity in the control sample.

Proteinase pH optimum studies were carried out with the following buffers: sodium citrate (pH 3.5-6), phosphate (pH 7-9), glycine-NaOH (pH 9 and 10), and Na_2HPO_4-NaOH (pH 10 and 11). Subsequently, proteinase determinations were conducted in 0.1 M sodium phosphate buffer at the appropriate pH optimum.

Proteinase and Protein Assays

General proteinase

General protease activity was determined by using sulfanilamide-azocasein as a substrate as described by Johnston et al. (1995) with modification. Ten µl of midgut extract were added to 0.5 ml reaction buffer and pre-incubated 5 min at 30 °C. The reaction was started by the addition of 0.2 ml of 1% azocasein, incubated at 30 °C for 1 hr, and stopped by adding 0.4 ml of 20% acetic acid. The solution was centrifuged at

10,000 rpm for 5 min, 1 ml of 1N NaOH was added and absorbance was measured at 440 nm.

Aminopeptidase

Aminopeptidase activity was measured using leucine p-nitroanilide (LPNA) at a final concentration of 2 mM (20 µl of 0.1 M LPNA in dimethylsulfoxide) (Lenz et al., 1991). The assay was started by addition of 1 ml reaction buffer containing 2.0mM substrate to 10 µl midgut or ovary extract. After 30 min incubation at 30 °C, the reaction was stopped by addition of 0.5 ml 20% acetic acid and enzyme activity was measured by monitoring p-nitroaniline at 410 nm.

Trypsin and chymotrypsin

Trypsin-like activity was determined using 1.0 mM tosyl-L-arginine methyl ester (TAME) or 2.0 mM benzoyl-DL-arginine-p-nitroaniline (BApNA) as substrates. Chymotrypsin-like activity was measured with 1.0 mM benzoyl L-tyrosine methyl ester (BTEE) or 0.1 mM BTpNA as substrates, based on the methods described by Christeller et al. (1989). For TAME and BTEE hydrolysis, the 1 ml reaction buffer containing 1 mM substrate was added to 10 µl midgut or ovary extract, incubated at 30 °C for 30 min, and absorbance monitored at 247 nm for TAME and at 256 nm for BTEE. For BApNA and BTpNA hydrolysis, the reaction was started by the addition of 1 ml of reaction buffer containing substrate to the midgut or ovary extract. Incubation was at 30 °C for 30 min. The reaction was stopped by addition of 0.5 ml 20% acetic acid, and monitored at 410 nm.

Carboxypeptidase A and B

Carboxypeptidases A and B activities were assayed using hippuryl-DL-phenyllactic acid (HPLA) and hippuryl-L-arginine (HA) as substrates, respectively (Houseman & Downe, 1981). The reaction was started by the addition of 1 ml of reaction buffer containing 1 mM HPLA or HA, and incubated 30 min at 30 °C and the reaction product monitored at 254 nm.

Assays were carried out in triplicate. Blanks prepared by addition of boiled extract or reaction buffer were used to account for the spontaneous breakdown of substrate. For nanomolar calculations, the molar extinction coefficients of 8800 at 410 nm (BApNA, BTpNA, and LPNA), 540 at 247 nm (TAME), 964 at 256 nm (BTEE), 280 at 254 nm (HPLA), and 360 at 254 nm (HA) were used (Lenz et al., 1991). The unit of molar extinction coefficient is $l\ mol^{-1}\ cm^{-1}$.

Preliminary experiments showed that proteinase activities increased with increasing of temperature from 25 to 37 °C using amidolytic substrates. For comparative purposes, a temperature of 30 °C, was adopted for the determination of proteinase activities in this study.

Protein concentrations were determined using the Bio-Rad Protein Assay system (Bio-Rad, Inc. Richmond, CA) with IgG as a standard. Enzyme activities are reported as nmol/min/organ except for azocasein where activity is reported as abs/min/organ. For *in vitro* inhibition and pH optima studies, the specific activity is recorded as nmole/min/mg protein or abs/min/mg protein.

Differences in mean proteinase activities of controls and treatments were analyzed by Tukey's HSD test after the GLM procedure (SAS Institute, 1989).

Results and Discussion

Proteinase Activities in Female *A. suspensa*

Table 1 shows the substrates used and the specific activities determined in the midgut. Trypsin, aminopeptidase, and general proteinase activities were detected in the midguts of newly emerged *A. suspensa*. These activities increased during first 4 d and appeared to be related to protein concentration in midgut (Fig. 5-1). From day 5 to 6 after emergence, proteinase activities decreased and then increased and decreased at daily intervals.

Ovarian proteinase activities were not detectable in the first 3 d and increased gradually with ovarian development (Fig. 5-2). Endoproteinase activities determined using BApNA or BTpNA reached maximal level when the ovary was near maturity, around day 7, and maintained this level during the oviposition period from day 7- 10; leucine aminopeptidase activity reached maximal level about 2 d later than endoproteinase, and was around 2 times higher than endoproteinase activity when the ovary matured. Table 5-2 shows the proteinase activities determined in the ovary of 7-d-old *A. suspensa* using different substrates.

Because the activity of chymotrypsin determined using BTpNA in the midgut was so low, it was difficult to quantify these activities in the midgut samples and no pesticide experiments on this enzyme activity in the midgut were conducted. For carboxypeptidase A and B, the hydrolysis products of the substrates HA and HPLA were measured at 254 nm, and it is difficult to provide individual crude protein extract blank. The carboxypeptidase data in Table 5-1 were produced from pooled midguts.

Usually, trypsin-like proteinases are more predominant than aminopeptidases in the gut of insects (Baker, 1982). However, our results and results reported by Baker (1982) on *Sitophilus* weevils showed that aminopeptidase activity was higher than trypsin activity using amidolytic substrates. Similar results were also reported in the midgut of the plant-feeding dipteran, *Rhynchosciara americana* (Wiedemann) (Terra et al., 1979), the horn fly, *Haematobia irritans* (L.) (Hori et al., 1981), and some lepidopterans such as *H. zea* (Lenz et al., 1991) and *Lymantria dispar* (Valettis, 1995).

Trypsin and chymotrypsin activities were different when different synthetic substrates were used. That is, esterolytic activities determined using TAME and BTEE were higher than amidolytic activities using BApNA and BTpNA (Table 5-1). Since the enzyme source used in determination was crude protein extract, TAME and BTEE could have been hydrolyzed by general esterases which may exist in crude extracts, and the hydrolysis of these ester substrates can also be catalyzed by many carboxypeptidases (Sarath et al., 1990).

pH Optima and Inhibition of Digestive Proteinases

Midgut proteinases

Proteinase activities in the midgut relative to pH are shown in Fig. 5-3A-C. Trypsin and general proteinase activities exhibited an optimum pH around 8 and 9, respectively. Aminopeptidase showed highest activity at pHs, 7.5 and 9.0. The trypsin activity pH optimum was comparable to that reported in many Dipteran species such as *H. irritans* (Hori et al., 1985) and *S. calcitrans* (Schneider et al., 1987; Moffatt & Lehane, 1990), but different from that in Lepidopteran larvae in which trypsin had high alkaline pH optimum, above 10 (Johnston et al., 1995, Novillo et al., 1997). In this study, the hydrolysis of BApNA with midgut extract was maximal at pH 8 in 0.1 M sodium phosphate buffer when a discontinuous buffer system between pH 5 and 10 was used (Fig. 5-3A). The optimum pH value may be changed, of course, with different buffer systems (Lee & Anstee, 1995).

Table 5-3 shows that 1 mM phenylmethylsulphonyl fluoride (PMSF) reduced trypsin activity (BApNA) around 70%, chymotrypsin activity (BTpNA) 67%, and 0.1 mM tosyl-L-lysine chloromethyl ketone (TLCK) inhibited trypsin activity 76%. General proteinase activity was inhibited an average of 88% and 24% by 1 mM PMSF and 0.1 mM TLCK, respectively. Methamidophos (1mM), an oxon organophosphorous pesticide, inhibited trypsin activity about 50%, but did not inhibit chymotrypsin (Table 5-3). Methamidophos appeared to be able to differentiate between trypsin and chymotrypsin activity, but more data are needed on this point. At 1 x 10^{-3} M, sodium tetraborate and imidacloprid did not inhibit proteinase activities *in vitro* using amidolytic substrates (Table 5-4). However, 1 x 10^{-3} M imidacloprid inhibited trypsin activity about 50% using the esterolytic substrate, TAME (Table 5-4).

To classify the type of insect proteinase, optimum pH, its sensitivity to specific substrates and inhibitors, and its similarity to well-characterized proteinases are commonly used (Wolfson & Murdock, 1990). BApNA, a substrate for trypsin-like proteinases, may also act as substrate for cysteine endoproteinases (Novillo et al., 1997). We excluded the presence of this type of enzyme in the midgut extract of *A. suspensa* because: 1) *A. suspensa* proteinase activities with BApNA and azocasein at pHs 5 and 6 were much lower than that at alkaline pH (Fig. 5-3A). 2) *A. suspensa* proteinase activities were inhibited by PMSF, a serine specific inhibitor, and by TLCK, a trypsin specific inhibitor, but were not inhibited by iodoacetamide (IAA), a cysteine proteinase inhibitor (Ortego et al., 1996) (Table 5-3). These results indicated that, like most other dipteran and all phytophagous lepidopteran species, the endoproteinase digestive enzymes in *A. suspensa* are serine proteases and trypsin-like enzymes are predominant (Sharma et al., 1984; Schneider et al., 1987; Johnston et al., 1995; Ortego et al., 1996).

Ovary proteinases

Figure 5-4A-C shows the relation of pH and *A. suspensa* ovary proteinase activities. As in the midgut, aminopeptidase activity in the ovary was highest at a pH around 7.5-8 when using LPNA as substrate. For both BApNA and BTpNA as substrates, ovary proteinase showed two pH maxima; one around pH 4.5, another around pH 8-9.

The effect of some inhibitors and activators on the activities of ovary proteinases is shown in Table 5-5. Except for $CaCl_2$ which enhances the hydrolysis of both BApNA and BTpNA, the proteinase activating and inhibiting compounds tested did not affect endoproteinase activities using BApNA and BTpNA as substrates.

As in *A. suspensa* midgut, ovary aminopeptidase activity was inhibited by the specific inhibitors, L-leucine chloromethyl ketone (LCK) and phenathroline when LPNA was used as substrate, but was not affected by EDTA, mercaptoethanol, and serine proteinase inhibitors (Table 5-5). Houseman & Downe (1981) reported that aminopeptidase in the midgut of *R. prolixus* was inhibited by calcium, EDTA, and mercaptoethanol. In the midgut of the Gypsy moth, the activity of aminopeptidase was inhibited slightly by diisopropylfluoro phosphate (DFP) and PMSF, both serine specific inhibitors (Valaitis, 1995).

Unlike the midgut, endoproteinase(s) in *A. suspensa* ovary was unlike either serine or cysteine proteinase and has its own special properties. Even though it can hydrolyze both BApNA and BTpNA at alkaline pH, but it is insensitive to serine specific inhibitors such as PMSF and TLCK (Table 5-5), it could not be classified as a serine proteinase. However, similar to proteinases in the eggs of other insects (Kageyama et al., 1981; Ribolla et al., 1993) it showed high activity at acidic pH 4.5 (Fig. 5-4A) when BApNA was used as substrate; this activity was increased by 2.0 mM calcium ion. These properties are similar to cathepsin-like cysteine proteinases (Barrett et al., 1982; Bond & Butler, 1987). However, *A. suspensa* ovary proteinase did not hydrolyze azocasein and was not affected by common inhibiting or activating compounds which are usually recognized as specific to cysteine proteinase such as E-64 and IAA (inhibitors) or activator such as mercaptoethanol (Barrett, 1994). Also, *A. suspensa* ovary proteinase hydrolyzed the synthetic substrate such as BTpNA, a specific substrate of serine proteinase (Applebaum, 1985). Based on the properties of high activity in 2 different pH regions and substrate and inhibitor responses, there may be more than one endoproteinase or one enzyme with multiple active sites in *A. suspensa* ovary.

Since enzyme source used in this experiment was crude extract, there may be many interaction factors during enzyme determination. In order to determine accurate properties of these enzymes, the characterization of pure enzyme(s) needs to be conducted.

Effect of Pesticides on Proteinase Activities in the Midgut and the Ovary

Midgut proteinase

Midgut proteinase activities of *A. suspensa* increased during first 4 days after emergence (Fig. 5-1). When newly emerged flies were treated with sodium tetraborate or imidacloprid by feeding for 24 hr, midgut proteinase activities were inhibited to different degrees in the first few days after treatment (Fig. 5-5A-C and Fig. 5-6A-C). For sodium tetraborate treated flies, proteinase activities were not different from controls at the 0.2% concentration, and when concentration was increased to 0.5%, all proteinase activities were inhibited significantly ($P < 0.05$) for the first 4 d after treatment (Fig. 5-5A-C). For imidacloprid treated flies, trypsin–like and general proteinase activities were only reduced from day 2 to 4 after treated with 1.0mg/l imidacloprid, and the reduction is less than in 0.5% sodium tetraborate treated flies. The activities of aminopeptidase and other proteinases in the flies treated with 0.5 mg/l or 1.0 mg/l imidacloprid were not significantly different from that of controls (Fig. 5-6A-C).

According to Christopher & Mathavan (1985), synthesis and secretion of digestive enzymes in insects appear to be regulated by three possible mechanisms, neural, hormonal, and secretogogue (secrection of digestive enzymes was stimulated by ingested food). Proteinase synthesis in the midgut of Medeira cockroach, *Leucophaea mederae* (Fabricius) (Engelmann, 1969) and *Glossina morsitata* (Gooding, 1974), is secretogogue. In *Catopsilia crocale*, hormones may play a major role in the regulation of digestive enzyme secretion (Christopher & Mathavan, 1985). For *A. suspensa*, high proteinase activities in the midgut of newly emerged adult female indicate that digestive proteinases were not secretogogue and may be regulated by hormone or neurosecretory system.

Rao & Fisk (1965) reported that trypsin activity in female *Nauphoeta cinerea* (Olivier) was related to ovarian development. A similar result was also found in female *Aedes aegypti* (Briegel & Lea, 1979). The *in vitro* inhibition results in Tables 5-3 and 5-4 indicate that sodium tetraborate and imidacloprid did not interact with proteinases themselves. The decrease of proteinase activities in the midgut of female *A. suspensa* treated with sodium tetraborate and imidacloprid may result from the interaction of these two chemicals with hormones and/or neurosecretory system which regulate proteinase synthesis/or secretion.

Ovary proteinases

The *in vitro* sodium tetraborate and imidacloprid effects on ovarian proteinase activities in *A. suspensa* are shown in Figure 5-7A-C. Both endoproteinase and exoproteinase activities were reduced to different degrees by imidacloprid and sodium tetraborate. For imidacloprid-treated flies, proteinase activities in the ovary of 7-d-old female *A. suspensa* were reduced to the similar degrees with different substrates at certain concentration, and the reduction of activities was proportional to treatment concentration. For sodium tetraborate treated flies, BApNA and LPNA proteinase activities were reduced more than BtpNA proteinase activity. In flies treated with 0.5% sodium tetraborate, both endoproteinase determined using BApNA and BTpNA and leucine aminopeptidase activities were reduced significantly, around 60-90%.

According to the pH optima and responses to specific substrates and inhibitors, the endoproteinases in the midgut of female *A. suspensa* were demonstrated to be serine proteinase with trypsin-like proteinase primary. Exoproteinases were detected as aminopeptidase and carboxypeptidase A&B. The trypsin, aminopeptidase, and general proteinase activities were not affected *in vitro* by sodium tetraborate and imidacloprid at 1×10^{-3} M level. However, the activities were reduced 20-80% at days 1 to 4 after treatment when flies were fed with 0.5% sodium tetraborate for 24hr. For imidacloprid treated flies, only trypsin and general proteinase activities were reduced from days 2 to 4 after 24hr feeding with 1.0mg/l concentration.

Endoproteinases in the ovary of *A. suspensa* were different from that in the midgut. When flies were treated as newly emerged adults with sodium tetraborate (0.2 & 0.5%) and imidacloprid (0.5 & 1.0mg/l) by feeding for 24hr, proteinase activities in the ovary of 7-d-old *A. suspensa* were reduced based on concentration with greater reduction in 0.5% sodium tetraborate treated flies.

Table 5-1. Proteinase activities in the midgut of 6-d-old female *A. suspensa.*

Enzyme	Substrate	pH	Activity (nmol/min/mg protein)
Trypsin	BApNA	8.0	20.5 ± 0.7
	TAME	8.0	604.9 ± 14.5
Chymotrypsin	BTpNA	8.0	3.3 ± 0.1
	BTEE	8.0	83.0 ± 14.7
Aminopeptidase	LPNA	7.5	197.2 ± 2.7
Carboxypeptidase A	HPLA	7.5	125.2 ± 31.8
Carboxypeptidase B	HA	7.5	92.6 ± 19.6
General proteinase	Azocasein	9.0	0.1 ± 0.0004 [a]

[a] Activity was expressed as absorbance of azocasein hydrolyzed/min/mg.

Table 5-2. Proteinase activities in the ovary of 7-d-old female *A. suspensa*.

Enzyme	Substrate	pH	Activity (nmol/min/mg protein)
Endoproteinase	BApNA	8.0	2.7 ± 0.04
	BTpNA	8.0	2.4 ± 0.2
Aminopeptidase	LPNA	7.5	11.2 ± 0.3

Table 5-3. *In vitro* inhibition of midgut proteinase activities of 4-d-old female *A. suspensa*.

		Inhibition (%)		
Inhibitor	Concentration	Trypsin (BApNA)	Chymotrypsin (BTpNA)	General protease (Azocasein)
PMSF	1 x 10^{-3} M	69.8 ± 6.8	66.7 ± 8.2	87.8 ± 3.5
TLCK	1 x 10^{-4} M	75.6 ± 2.0	0	24.3 ± 13.5
Methamidophos	1 x 10^{-3} M	54.5 ± 2.6	0	53.3 ± 3.5
IAA	1 x 10^{-3} M	0	0	0

Table 5-4. *In vitro* pesticide effects on proteinase activities in the midgut of 4-d-old female *A. suspensa*.

		Activity (nmol/min/mg protein)		
Enzyme	Substrate	Control	Sodium tetraborate (1 x 10^{-3} M)	Imidacloprid (1 x 10^{-3} M)
Trypsin	BApNA	31.2 ± 0.8	29.8 ± 2.8	28.8 ± 7.4
	TAME	870.7 ± 89.4	778.1 ± 78.6	352.0 ± 54.5
Chymotrypsin	BTpNA	6.8 ± 0.8	7.4 ± 0.8	6.2 ± 1.6
Aminopeptidase	LPNA	262.8 ± 10.4	268.7 ± 4.8	248.6 ± 1.6
General protease	Azocasein	0.03 ± 0.001	0.04 ± 0.003	0.03 ± 0.004[a]

[a] Activity was expressed as absorbance/min/mg protein.

Table 5-5. *In vitro* effect of various of potential proteinase inhibiting or activating compounds on the hydrolysis of BApNA, BTpNA, and LPNA by ovary homogenate from 7-d-old female *A. suspensa.*

		Relative activity [a]		
Compounds	Conc. (M)	BApNA	BTpNA	LPNA
Sodium tetraborate	1×10^{-3}	102	106	97
Imidacloprid	1×10^{-3}	108	107	105
PMSF	1×10^{-3}	95	102	--
TLCK	1×10^{-4}	96	103	--
E-64	2×10^{-5}	105	94	--
IA	5×10^{-3}	109	98	--
IAA	5×10^{-3}	103	111	--
DTT	4×10^{-3}	107	112	93
Mercaptoethanol	5×10^{-3}	113	101	108
EDTA	4×10^{-3}	106	105	88
$CaCl_2$	2×10^{-3}	174	148	121
LCK	1×10^{-4}	--	--	67
Phenanthroline	1×10^{-3}	--	--	63

[a] Percent of control.

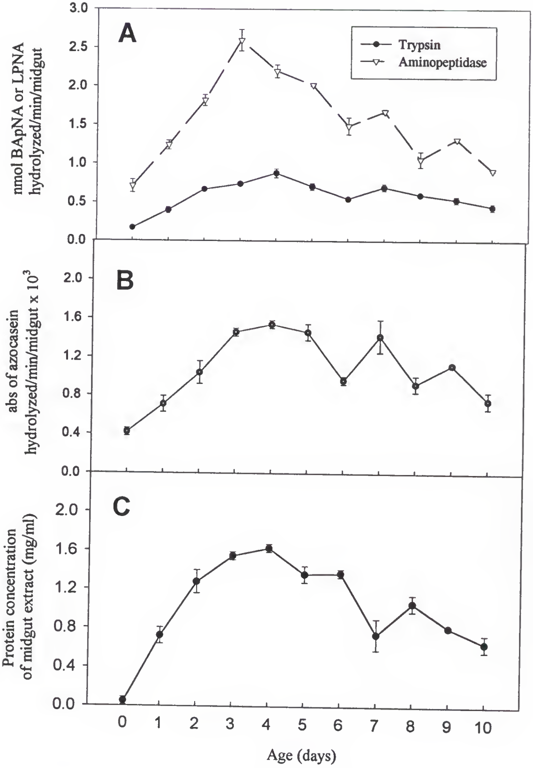


Figure 5-1. Time course of proteinase activities and protein concentration in the midgut of female *A. suspensa.* A: Trypsin and aminopeptidase; B: General proteinase; C: Protein concentration.

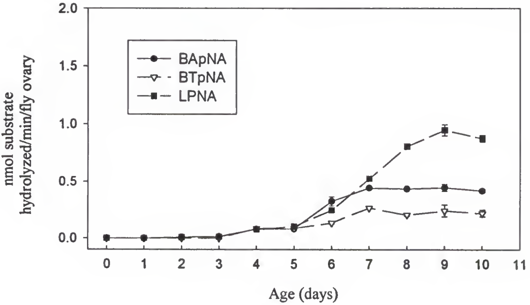


Figure 5-2. Time course of ovary proteinase activities of *A. suspensa* BApNA & BTpNA: endoproteinase, LPNA: aminopeptidase.

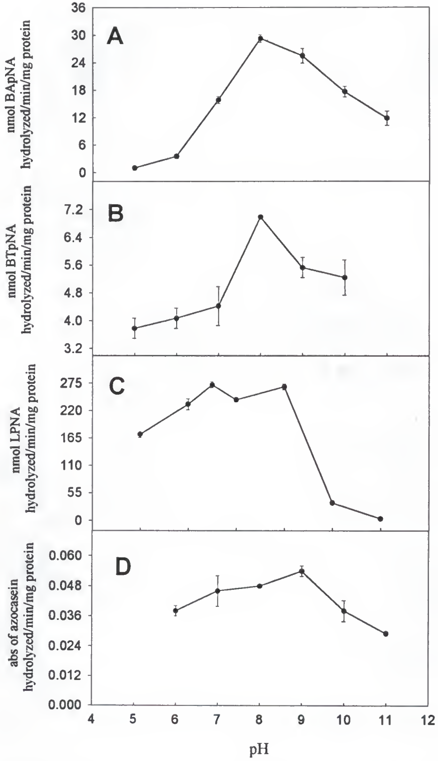


Figure 5-3. pH and proteinase activities in the midgut of female *A. suspensa*
A: Trypsin; B: Chymotrypsin; C: Aminopeptidase; D: General proteinase.

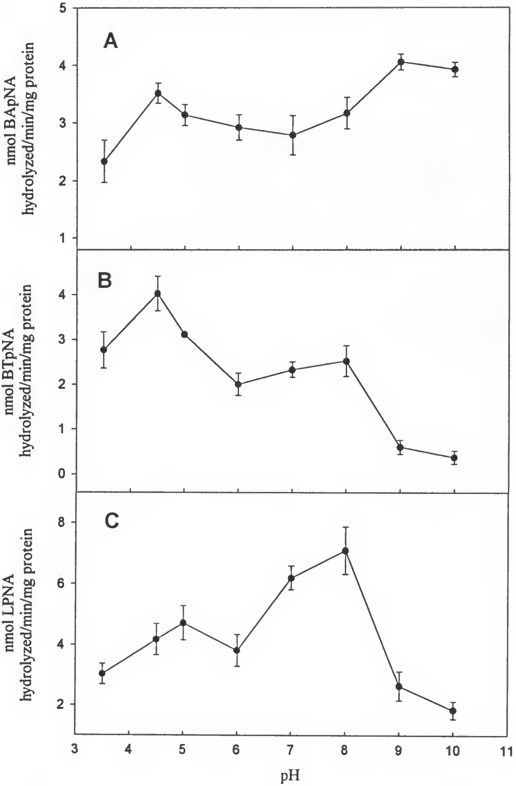


Figure 5- 4. pH and proteinase activities in the ovary of 7-d-old *A. suspensa*, A&B: Endoproteinase (BApNA & BTpNA); C: Aminopeptidase(LPNA).

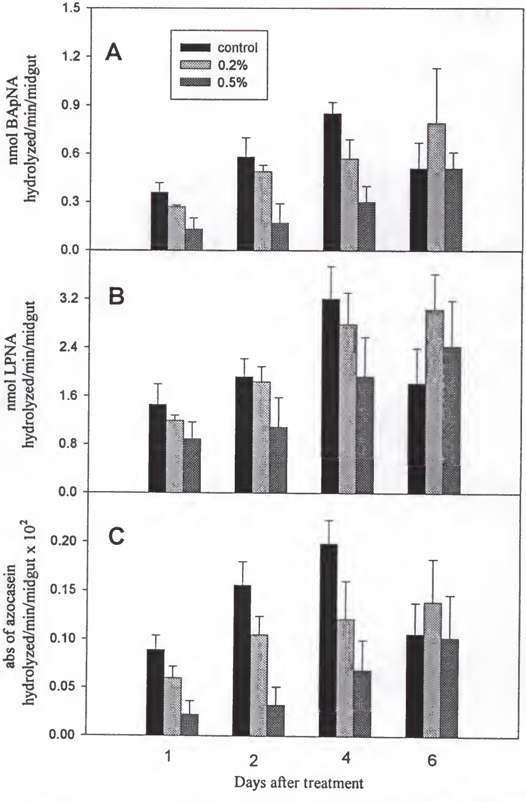


Figure 5-5. Midgut proteinase activities of female *A. suspensa* treated as newly emerged adults with sodium tetraborate for 24hr. A: Trypsin (BApNA); B: Aminopeptidase (LPNA); C: General proteinase(Azocasein).

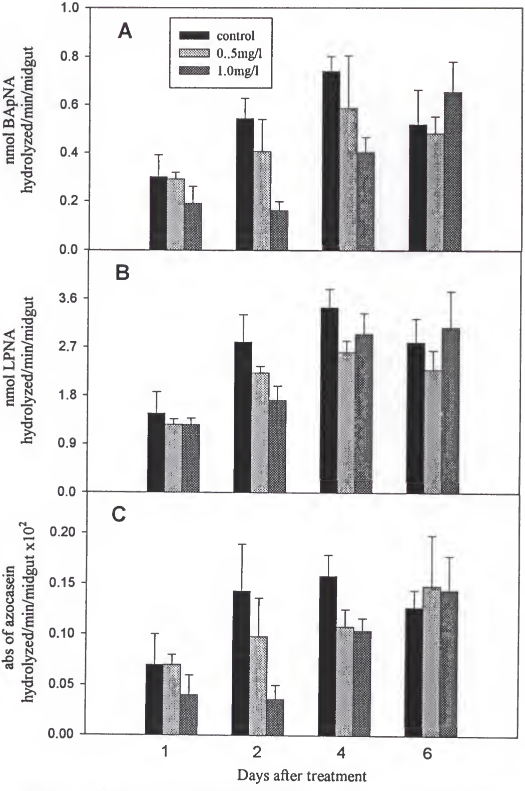


Figure 5-6. Midgut proteinase activities of female *A. suspensa* treated as newly emerged adults with imidacloprid for 24hr, A: Trypsin; B: Aminopeptidase; C: general proteinase.

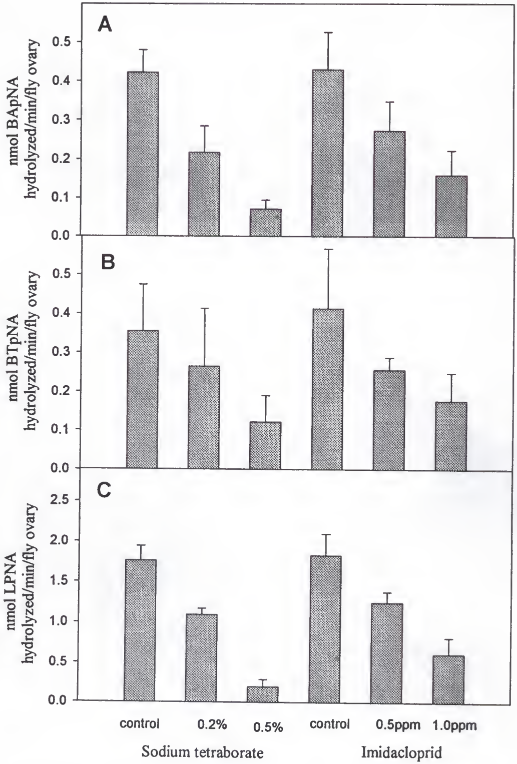


Figure 5-7. Ovarian proteinase activities in 7-d-old *A. suspensa* treated as newly emerged adults with sodium tetraborate or imidacloprid for 24hr, A&B: Endoproteinase; C: Aminopeptidase.

CHAPTER 6
EFFECT OF SODIUM TETRABORATE AND IMIDACLOPRID ON GENERAL ESTERASE ACTIVITIES IN *ANASTREPHA SUSPENSA*

Introduction

Esterases are a large and diverse group of enzymes and have a wide range of substrate specificity (Dary et al., 1990). Two types of esterases, JH esterase (JHE) and esterase 6 (EST 6), have been reported to be involved in the reproduction of insects (Richmond et al., 1980; Shapiro et al., 1986; De Kort & Granger, 1996).

JHE, a nonspecific carboxylesterase, is produced in both the fat body and the ovary in insects (McCaleb & Kumaran, 1980; Shapiro et al., 1986). It is thought to play a role in the regulation of JH titer during metamorphosis and the adult reproductive cycle (Hammock, 1985; Venkatesh et al., 1988; Zera et al., 1993). As previously described in chapter 2, JH is an important factor in the regulation of egg development (Cymborowski, 1992; Hoffmann, 1995). Shapiro et al. (1986) have shown in *Aedes aegypti* that JH levels and JHE activity were inversely correlated after a blood meal during egg development. Presence of JHE has been correlated in many other species with the degradation of JH during egg development of insects (Tanaka, 1994; De Kort & Granger, 1996; Cusson & Delisle, 1996). In *Acheta domesticus* (Linnaeus), the extent of ovarian development and egg production were significantly reduced when recombinant JHE was repeatedly injected into female insects (Bonning et al., 1997).

EST 6, a nonspecific carboxylesterase, is produced in the male reproductive tract and is transferred into the female during mating (Sheehan et al., 1979). The role of EST 6 on the reproduction of insects has been extensively studied in *Drosophila melanogaster* (Sheehan et al., 1979; Richmond et al., 1980). The study results showed that *D. melanogaster* females mated by males with high active EST 6, mated again sooner and produced more eggs (Richmond et al., 1980; Gilbert et al., 1981).

In this study, the effect of sodium tetraborate and imidacloprid on the general esterase in the abdomen and whole body of both male and female *A. suspensa* were examined.

Materials and Methods

Chemicals and Equipment

Methamidophos was obtained from Bayer Agriculture Division (Valrico, FL), imidacloprid from Miles Inc. (Kansas City, MO), paraoxon from Chem-Service (West Chester, PA), sodium tetraborate from Fisher Scientific (Fair Lawn, NJ), and other chemicals were obtained from Sigma Chemical Co., Ltd. (St. Louis, MO). Spectrophotometric measurements were made using a Shimadzu UV-2410 PC spectrophotometer (Shimadzu, Columbia, MD).

Insects

A. suspensa were supplied as 9 d old pupae from the Florida Department of Agricultural and Consumer Services, Division of Plant Industry, Gainesville, FL. Flies which emerged over 4 hr were placed in 30.5 cm^3 cages (Bio Quip, Inc., Gardero CA), approximately 1500 flies per cage, and were treated immediately with 0.2% and 0.5% sodium tetraborate and 0.5 mg/l and 1.0 mg/l imidacloprid by feeding for 24 hr. Control was treated in the same way except that no pesticides were added to their food. Each treatment was replicated 3 times.

Esterase Preparation

Male and female flies from control and treatments were sampled 24, 48, 96, and 144 hr after treatment and stored at - 40 °C until used. Flies in the time course experiment were collected daily from day 0 to day 10 after emergence. Abdomens were removed and placed in sodium phosphate buffer (0.1 M, pH 7.5, 10 abdomens/500 μl) by sex. Whole body flies were stored in same buffer (5 flies/500 μl) by sex. Samples were homogenized manually in micro-centrifuge tubes and centrifuged at 10,000 rpm for 15 min. The supernatant was aliquoted for enzymatic activity and protein assays. All procedures were performed on ice or at 4 °C.

Esterase Assay

Non-specific esterase activity was determined using the α-naphthylacete (α-NA) assay as described by Lindroth et al. (1990). Crude extract solution (10 μl) was added to 0.5 ml of 0.1 M, pH 7.5 sodium phosphate buffer containing 0.1 mM α -NA and incubated at 32 °C. Ten minutes later, 1 ml of the stop solution, 0.02% fast blue B and 0.75% SDS, was added into reaction mixture. After 10 min of color development by coupling the fast blue B with released naphthol, absorbance at 600 nm was recorded and converted to concentration of α-naphthol using a standard curve. Inhibition studies were conducted by addition of specific inhibitors to abdomen or whole body extracts and incubation for 20 min at 32 °C before substrates were added. Enzyme activities are reported as nmol α-NA hydrolyzed/min/organ.

Statistical Analysis

Data were analyzed using the general linear model (GLM) procedure. Significance differences among control and treatments ($P = 0.05$) were tested using Tukey's HSD test (SAS Institute, 1989).

Results and Discussion

General Esterase Activity in *A. suspensa*

The time course of general esterase activities in the abdomen and whole body of male and female *A. suspensa* are shown in Fig. 6-1A and Fig. 6-1B, respectively.

Esterase activity in the abdomen and whole body of both sex flies increased after adult emergence. Esterase activity in the female abdomen doubled 6 -7 d after emergence and decreased slightly when oviposition began; in the whole body, the esterase activity decreased slightly in the first day after eclosion, increased slightly during next 5 days, and then decreased again when ovary was approached maturity at day 7. This result was different from that reported by Venkatesh et al. (1988) on adult cabbage looper, *Trichoplusis ni*, in which both general esterase and JHE activities in the plasma of female showed a sharp decline from emergence to 1 d old and then only slight change to 10 d.

Unlike females, male esterase activity doubled in the whole body assay and increased in the abdomen 7 times from emergence to 9-10 d old flies. This agrees with the results reported by Sheehan et al. (1979) and Richmond et al. (1980) on *Drosophila melanogaster* in which EST 6 activity determined using α-NA as substrate in the adult male was 5 or more times higher than in any other developmental stage or in the adult females. These results indicate that general esterase determined in the abdomen of male flies was comparable to EST 6. In male flies, general esterase activity in the abdomen was about one-third of that in the whole body. In female flies, this proportion was 1(abdomen) to 8 (whole body).

Inhibition of General Esterase Activity in *A. suspensa*

General esterase activities in the abdomen and the whole body of male and female *A. suspensa* were inhibited *in vitro* about 10- 40% by sodium tetraborate and imidacloprid; inhibition caused by sodium tetraborate is slightly less than imidacloprid

(Table 6-1). General esterase in the abdomen and whole body of both males and females were equally susceptible to PMSF; general esterases in the whole body were slightly more sensitive to methamidophos than that in the abdomen of both male and female flies. This may be understandable since organophosphate is a more potent inhibitor of cholinesterase, which is mainly produced in the thorax part of insects, than carboxylesterase (Bigley & Plapp, 1960). Paraoxon showed powerful inhibition on all determined general esterases; methamidophos showed higher inhibition on whole body esterases than abdomen esterases in both males and females, e.g. approximately 60% inhibition of general esterases in the whole body of females was obtained at 10^{-5} M, but the abdomen esterase was only inhibited around 25% at the same concentration. These results indicate that general esterases determined in the abdomen and whole body of *A. suspensa* have different properties and they may belong to different subclasses of esterases.

Venkatesh et al. (1988) found that general esterase determined using α-NA as substrate in *T. ni* is much more sensitive to DFP than JHE. Similarly, in *Manduca sexta,* Jesudason et al. (1990) reported that the activities of JHE and α- NAE were inhibited 12% and 83% by $1x10^{-4}$ M DFP, respectively.

Pesticide Effects on General Esterase

General esterase activity in the female abdomen increased after newly emerged flies were treated with 0.5% sodium tetraborate by feeding for 24 hr; whereas, general esterase activity in the whole body of female did not change for 6 d after treatment (Fig.

6-2A and C). As speculated above, esterase in the female abdomen is only a small part of that in the whole body, and they may differ from each other.

Koopmanschap & De Kort (1989) reported that the main carboxylesterase activity in the hemolymph of *Locusta migratoria* hydrolyzes JH as well as α- and β-NA. Similarly, the JHEs from *Galleria mellonella* (Rudnicka & Kochman, 1984) and *T. ni* (Hanzlik & Hammock, 1987) also showed some activity with α-NA as a substrate. Based on this information and the inhibition study as previously described, general esterase activity determined in the abdomen of female *A. suspensa* may represent, or partially represent, JHE activity. If this were true, the increase in esterase activity after feeding sodium tetraborate may be related to a decrease of JH which may play a role in the regulation of egg development in *A. suspensa*. Although stimulation of yolk protein synthesis by a JH analog was not observed by Handler & Shirk (1986), a possible effect of JH on ovarian development in *A. suspensa* may be revealed by further study, in particular *in vitro* culture of ovaries (Handler, 1997). Further study on the determination of JHE using more specific methods, e.g., radioimmunoassay, also need to be conducted.

In male *A. suspensa*, general esterase activities in the abdomen and the whole body were reduced from 4 to 6 d after 24 hr feeding 0.5% sodium tetraborate with greater reduction in the abdomen (Fig. 6-2B and D). This reduction of esterase in male flies may result in deficient male mating and finally affect egg hatch. This result was comparable with the results obtained in Chapter 3 in which egg hatch was reduced when male flies were treated immediately after emergence with 0.5% sodium tetraborate for 24 hr. When flies were treated with 0.5 or 1.0 mg/l imidacloprid, general esterase activities in the abdomen of females and the whole body of both male and female flies were not affected (Fig. 6-3A,C and D); esterase activity in the male abdomen was reduced 20- 40% from 4 to 6 d after treatment with 1.0 mg/l imidacloprid (Fig. 6-3B).

General esterase activity against a-NA was determined in the abdomen and whole body of *A. suspensa*. Based on different responses to various inhibitors, the esterases in the abdomen and whole body had different properties and may be different enzymes. When flies were treated as newly emerged adults with 0.5% sodium tetraborate and 1.0mg/l imidacloprid for 24hr, general esterase activity in the abdomen of both males and females were reduced at days 4-6 after treatment with greater reduction in sodium tetraborate treated flies. General estarase activity in female abdomen was increased at days 4-6 after treatment when flies were fed with 0.5% sodium tetraborate. General esterase activity in the whole body of both males and females were not affected by these two chemicals after 24 treatment.

In the last two chapters, effects of sodium tetraborate and imidacloprid on the proteinase and general esterase activities of *A. suspensa* were detailed. The last sub-project will focus on the effects of these two chemicals on yolk protein synthesis and yolk protein gene transcription.

Table 6-1. Inhibition of general esterase activities in the abdomen and the whole body of male and female *A. suspensa.*

	Inhibition (%) at 1 ×10^{-5} M			
Compound	♀ abdomen	♀ whole body	♂ abdomen	♂ whole body
Sodium tetraborate	9.2 ± 11.4a	10.7 ± 3.2a	12.2 ±15.9a	5.5 ± 6.4a
Imidacloprid	27.2 ± 22.7a	43.9 ± 7.2a	17.2 ± 7.7a	27.4 ± 18.0a
PMSF	29.1 ± 15.5a	18.9 ± 8.2a	30.1 ± 14.2a	26.0 ± 15.7a
Methamidophos	25.2 ± 21.1b	63.0 ± 8.7a	15.1 ± 9.6b	38.6 ± 18.4ab
Paraoxon	94.2 ± 2.9ab	98.7 ± 1.2a	92.9 ± 0.9b	97.3 ± 2.7ab

Means within each row followed by the same letter are not significantly different (P = 0.05, Tukey's HSD test).

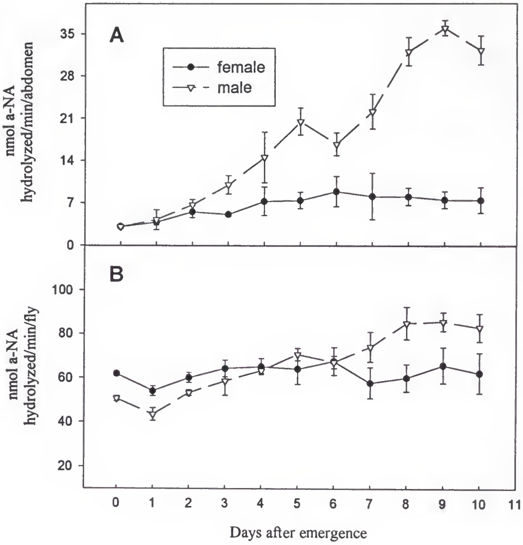


Figure 6-1. Time course of general esterase activities in the abdomen (A) and the whole body (B) of *A. suspensa.*

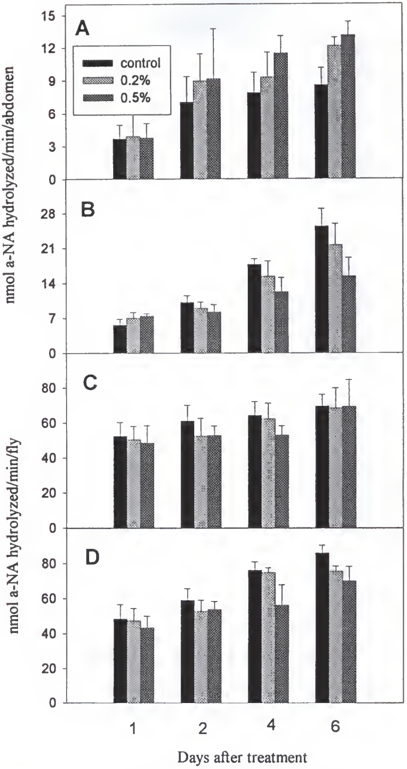


Figure 6-2. General esterase activities in the abdomen (A&B) and the whole body (C&D) of *A. suspensa* treated as newly emerged adults with sodium tetraborate for 24hr, A: Female; B: Male; C: Female; D: Male.

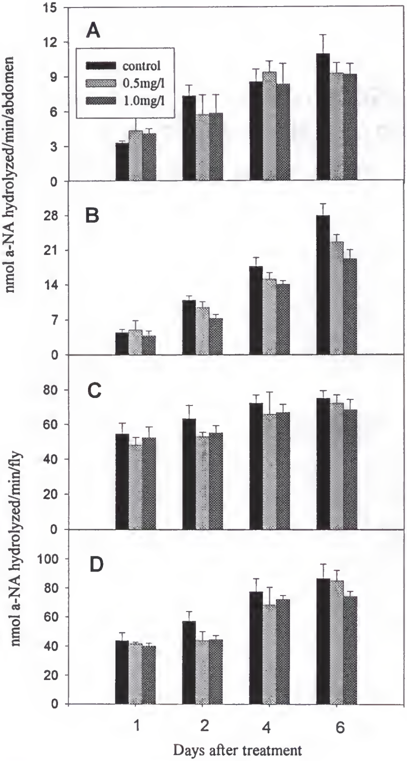


Figure 6-3. General esterase activities in the abdomen (A&B) and the whole body (C&D) of *A. suspensa* treated as newly emerged adults with imidacloprid for 24hr, A: Female; B: Male; C: Female; D: Male.

CHAPTER 7
EFFECT OF SODIUM TETRABORATE AND IMIDACLOPRID ON YOLK PROTEIN SYSTHESIS IN *ANASTREPHA SUSPENSA*

Introduction

Vitellogenin, the precursor of yolk protein (YP), has been recognized as an important protein during egg development in insects (Hagedorn & Kunkel, 1979). In most insects, this protein is synthesized in the fat body, secreted into the hemolymph, and selectively accumulated by the developing oocytes in which it is sequestrated as vitellin (Engleman, 1979; Hagedorn & Kunkel, 1979). Vitellogenin synthesis has also been found to occur in the ovarian follicular epithelium of many Diptera species (Brennan et al., 1982; Rina & Mintzas, 1988) and the fat body of some male insects (Lamy, 1984). In the stable fly, *Stomoxys calcitrans* (Houseman & Morrison, 1986; Chen et al., 1987), and the Caribbean fruit fly, *Anastrepha suspensa* (Handler, 1997), the ovary has been shown to be the sole site of yolk protein synthesis.

The process of vitellogenin synthesis, secretion, and uptake has been shown to be related to hormone stimulation (Handler & Postlethwait, 1978; Huybrechts & De Loof, 1982) and protein digestion (Bownes & Blair, 1986; Bownes et al., 1988). For most insects, vitellogenin synthesis is regulated either by JH or ecdysteroids (Izumi et al., 1994). In many Diptera, both ecdysteroids and JH have been reported to be involved in vitellogenin synthesis in the fat body and/or ovary (Hagedorn, 1985; Hardie, 1995). The involvement of diet and hormones in vitellogenin gene transcription has been further demonstrated in some insect species, e.g. *Drosophila melanogaster* (Bownes & Blair, 1986; Bownes et al., 1988), and *Musca domestica* (Agui et al., 1991; Adams & Gerst, 1992)

Yolk proteins of most higher Diptera such as *Sarcophaga, Calliphora, Phormia*, and *Lucilia* have similar molecular mass and exhibit evolutionary conservation in their structure (Huybrechts & De Loof, 1982). Yolk proteins characterized from 3 Tephritid species, *C. capitata* (Rina & Mintzas, 1987), *D. oleae* (Levedakou & Sekeris, 1987), and *A. suspensa* (Handler & Shirk, 1988), had a molecular weight around 45-55 kDa. The antibody to the purified 48 kDa YP from *A. suspensa* recognized all 3 YPs from *D. melanogaster* (Handler & Shirk, 1988), and *D. melanogaster* YP and *C. capitata* vitellogenin shared a high DNA and amino acid sequence homology (Rina & Savakis, 1991).

In this experiment, developmental and tissue specific vitellogenin synthesis, vitellogenin gene transcription, and pesticide effects on these processes were examined.

Materials and Methods

Insect Rearing and Treatment

A. suspensa were obtained as 9 d old pupae from the Florida Department of Agricultural and Consumer Services, Division of Plant Industry, Gainesville, FL. To evaluate the effect of sodium tetraborate and imidacloprid on vitellogenin synthesis, two groups of 8 pairs of newly emerged flies and one group of 8 male or female flies were set up; one group of paired flies was treated immediately with 0, 0.02, 0.05, 0.1, 0.2, and 0.5% sodium tetraborate or 0, 0.05, 0.1, 0.2, 0.5, and 1.0 mg/l imidacloprid by feeding for 24 hr. One group of single sex flies was treated immediately with 0.5% of sodium tetraborate or 1.0 mg/l imidacloprid. After 24 hr, single sex flies were paired with untreated flies. Another group of paired flies was treated with 0.5% of sodium tetraborate or 1.0 mg/l imidacloprid at different ages on days 0, 1, 3, and 5 after emergence . Each treatment was repeated 3 times. Controls were prepared in each test group by feeding food without pesticides. After 24 hr treatment, food with pesticides was removed and changed to normal food. Normal food and food with pesticides were prepared as described above. Female flies from control and each treatment were randomly collected on day 7 after emergence and kept at -40 °C until used. For the time course experiment of vitellogenin synthesis, flies were fed on normal food and female flies were collected daily to 7 d after emergence.

For the experiment to determine the effect of pesticides on vitellogenin gene transcription, flies were treated as follows. After flies emerged over 4 hr, three screened cages (30 x 30 x 30 cm) were prepared and approximately 1500 flies were put into each cage. One cage was treated immediately with 0.5% sodium tetraborate and another with 1.0 mg/l imidacloprid by feeding for 24 hr. The third cage was fed on normal food and served as control. Each experiment was repeated 3 times. Flies from control and treatments were collected 1, 3, 5, 7, and 9 d after treatment and kept at -70 °C until used for RNA extraction.

Preparation of Yolk Protein

Yolk protein from different tissues of flies was prepared according to the method of Mintzas & Kambysellis (1982). Dissected ovaries, fat bodies, and abdomens were rinsed with DDW and manually homogenized in 0.1 M sodium phosphate buffer, pH 7.5 (10 organs/0.5 ml). The supernatant was collected after 15 min centrifugation at 12,000 rpm and 4 °C, and dialyzed against 50% and 80% of ammonium sulphate solution overnight at 4 °C, respectively. After centrifugation at 12,000 rpm and 4 °C, for 15 min, the precipitate from the second dialyzed solution was collected and dissolved in 20 µl of 0.1 M sodium phosphate buffer.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS (0.1%)-polyacrylamide (10%) gel was prepared following Chen et al. (1978) with modification. Protein samples was denatured at 100 °C for 3 min in 0.1 M sodium phosphate buffer (pH 7.5) containing 2% SDS, 4% 2-mercaptoethanol and 0.002% bromophenol blue. Standard proteins (Bio-Rad, CA), lysozyme (18.5 kDa), soybean trypsin inhibitor (27.5 kDa), carbonic anhydrase (32.5 kDa), ovalbumin (49.5 kDa), bovine serum albumin (80 kDa), and phosphorylase B (106 kDa), were treated with SDS at the same time as samples to obtain a molecular weight estimation of vitellogenin. Electrophoresis was performed in Tris-glycine buffer (0.025 M, pH 8.6, 0.19 M glycerol) containing 0.1% SDS at a constant current of 5 mA per gel for 2.5 hr. After electrophoresis, gels was stained for protein in Coomassie brilliant blue for 1 hr and de-stained in 45% methanol and 10% acetic acid for 2.5 hr (Chen et al., 1978; Harnish & White, 1982).

Western Immunoblot Analysis

The immunoblot procedure was performed according to Handler & Shirk (1988) with modification. Precipitated vitellogenin samples were resolved on SDS-PAGE as described above and then electroblotted to nitrocellulose in transfer buffer (25 mM Tris, pH 8.3, 192 mM glycin, 20% methanol) at 20 V using a Transblot cell (Bio-Rad, Richmond, CA). After the transfer, the electroblot was blocked with 5% skim milk and incubated at 37 °C for 1.5 hr with shaking in fresh 5% milk in Tris buffered saline (20 mM Tris, pH 7.5, 0.5M NaCl) containing the first antibody (kindly provided by Dr. A. M. Handler, USDA, Gainesville, FL). The specific binding of the target protein with the serum was determined by visualizing the bands with an immunoblot color assay using a horseradish peroxidase-linked goat anti-rabbit IgG as the second antibody.

Preparation of RNA from Different Tissues

RNA extraction from various tissues of flies was performed following the procedure outlined in the RNA extraction kit instruction of the Life Technologies (Rockville, MD). Flies were inmobilized in - 17 °C freezer and dissected in DDW. Ovaries, fat bodies or abdomens were pulled out from dissected male and female flies, manually homogenized in 0.5 ml Trizol reagent (Gibco BRL, Gaithersberg, MD), and centrifuged for 15 min at 12,000 rpm at 4 °C. The supernatant was mixed with 0.1 ml of chloroform by vigorously shaking for 15 sec. After 15 min centrifugation at 12,000 rpm and 4 °C, the upper aqueous phase was transferred to a fresh tube, incubated with 0.25 ml of isopropyl alcohol for 10 min at room temperature for RNA precipitation. After 10 min centrifugation at 12,000 rpm and 4 °C, the gel-like RNA pellet was washed with 0.5 ml of 75% ethanol, air dried 10 min at room temperature, and redissolved in 40 µl RNase free water by incubating at 60 °C for 10 min.

DNA Probe Preparation and Estimation

cDNA of *A. suspensa* yolk protein (AsYP) was isolated from plasmid pUCAsYP (kindly provided by Dr. A. M. Handler, USDA, Gainesville, FL) by incubation at 37 °C for 2 hr with *Eco*RI, purified using 1.0% agarose gel and DNA purification column, and finally dissolved in 50 µl distilled de-ionized water. Random primed DNA labeling and estimation were carried out according to the manufacturer's protocols (Boehringer Mannheim). The labeling reaction was started by adding 1 µl Klenow enzyme in 19 µl water solution containing 2 µg denatured DNA template, 2 µl hexanucleotide mixture (10×), and 2 µl dNTP labeling mixture (10×), incubated at 37 °C for 2 hr, and terminated by adding 2 µl 0.2 M EDTA solution. Digoxigenin (DIG)-labeled DNA was precipitated at -70 °C for 30 min after adding 1 µl glycogen, 2 µl 4 mM LiCl, and 60 µl chilled ethanol. After quick thawing and 15 min centrifugation at 13,000 rpm, the DNA pellet formed, and then was washed with 70% ethanol, air dried, and re-suspended in 50 µl Tris buffer contained 0.1% SDS, pH 7.5. Estimation of DIG-labeled DNA was performed by comparing spot intensities of a serially diluted solution of the newly labeled experimental DNA probe with the labeled control DNA. The spot color was produced by color substrate, 45 µl nitroblue tetrazolium solution and 35 µl X- phosphate solution in 10 ml of detection buffer (0.1 M Tris-HCL, 0.1 M NaCL, and 0.05 M $MgCl_2$, pH 9.5), reacted with anti-DIG-alkaline phosphatase which specifically binds to DIG-labeled DNA.

Analysis of Yolk Protein RNA

RNA samples (2 µl) were denatured with 10 µl denaturing buffer containing 8.6% formaldehyde, 67% formamide, 5 mM sodium acetate, 1 mM EDTA, 20 mM morpholinopropansulphonate (MOPS), pH 7.0 for 10 min at 70 °C, cooled on ice, mixed with 2 µl loading buffer and resolved on 0.9% agarose MOPS gel containing 1.9% formaldehyde. After electrophoresis, RNA was electroblotted onto nylon membranes (Boehringer Mannheim) in transfer buffer, 25 mM sodium phosphate with pH 6.45, at 4 °C in an electroblotting chamber (Hoefer). After the transfer, the membrane was UV-cross linked in UV Stratalinker (Stratagene), pre-hybridized with hybridization buffer containing 50% formamide, 0.2% SDS, 0.1% n-lauroyl sarcosine, 2% casein, 5 × SSC (1 × SSC: 150 mM sodium citrate, 15 mM NaCl) for 1 hr at 42 °C, and hybridized with DIG-labeled DNA probe in the above buffer at 42 °C overnight. The hybridized membrane was washed 2 × 5 min at room temperature with 2 × SSC; 0.1% SDS and 2 × 20 min at 42 °C with 0.1 × SSC; 0.1% SDS. Specific RNA was probed with anti-DIG-alkaline phosphatase Fab fragment, developed with CSPD chemiluminescent substrate according to the manufacturer's protocols (Boehringer Mannheim), and exposed to X-ray film.

Results and Discussion

Developmental and Tissue Specificity of Yolk Protein and its RNA Accumulation

Figures 7-1 and 7-2 show time course of vitellogenin synthesis and tissue specificity of vitellogenin. Vitellogenin was detected in the ovary 3 d after emergence and a large increase in vitellogenin accumulation began around day 5. A protein with molecular size around 48 kDa was detected both in male and female abdomens as well as female fat body, which was in agreement with the result reported by Handler & Shirk (1988). In Mediterranean fruit fly, *Ceratitis capitata*, vitellogenins appeared in the hemolymph during the first day of the adult life and reached a maximum concentration during the fourth day (Rina & Mintzas, 1987).

Yolk protein gene transcription in different tissues of male and female *A. suspensa* was detected by Northern blotting using the cDNA probe derived from *A. suspensa* ovary mRNA (Fig. 7-3). No vitellogenin RNA accumulation was found in the abdomen of male flies and the fat body of female flies, and vitellogenin gene transcription was only detected in the ovary. This result was in agreement with the result reported by Handler (1997). He reported that AsYP gene transcription was limited to the ovary of female *A. suspensa* even though low level vitellogenin was detected in the fat body of both males and females (Handler & Shirk, 1986), since immunological probes (antiserum) used to detect vitellogenin might not be totally specific. In other Diptera species, e.g., *Dacus oleae* (Zongza & Dimitriadis, 1988), *Drosophila melanogaster* (Bownes & Reid, 1990), and *Musca domestica* (Agui et al., 1991), vitellogenin gene transcription occurs both in the ovary and the fat body.

Unlike the result reported by Handler (1997) who only showed one mRNA, in our experiment, 4 yolk protein mRNAs were detected from the ovary of *A. suspensa* (personal communication with Dr. Handler). This result is comparable with other Dipteran species in which 3, 5, and 4 vitellogenin genes were detected in *D. melanogaster* (Bownes, 1979), *A. aegypti* (Gemmill et al., 1986), and *C. capitata* (Rina & Savakis, 1991), respectively.

Effect of Pesticides on Yolk Protein Synthesis

When flies were treated immediately after emergence with various concentrations of sodium tetraborate by feeding 24 hr, yolk protein synthesis was only affected by 0.5% of sodium tetraborate (Fig. 7-4A), and when flies were treated at different ages from day 0 to day 5 with 0.5% of sodium tetraborate, all yolk protein synthesis was reduced regardless of fly age (Fig. 7-4B). If flies were treated with imidacloprid as above, yolk protein synthesis was not influenced whether flies were treated immediately after emergence with different concentrations up to 1.0 mg/l or treated with 1.0 mg/l at different ages (Fig. 7-5).

Bownes et al. (1988) reported that when *D. melanogaster* were starved from the time of eclosion, no vitellogenin synthesis occurred; very few vitellogenic oocytes were seen, and levels of YP gene transcription were reduced. Similarly, in *M. domestica*, when flies were fed with sucrose, vitellogenin level was very low, and increased after pulse feeding protein (Adames & Gerst, 1992). In *A. suspensa*, Nigg et al. (1995) showed that eggs were not produced when flies were maintained on only sugar.

Effect of Pesticides on Yolk Protein Gene Transcription

Figure 7-6 shows the effect of sodium tetraborate and imidacloprid on vitellogenin gene transcription. For the untreated flies, vitellogenin gene transcription began around day 3 and reached the highest level around day 5 after emergence, and mRNA accumulation continued after the ovary matured. When flies were treated immediately after emergence with 1.0 mg/l of imidacloprid by feeding for 24 hr, the pattern of yolk protein mRNA accumulation was the same as that of the control, which indicated that 1.0 mg/l of imidacloprid did not affect vitellogenin gene transcription in *A. suspensa*. However, when newly emerged flies were treated with 0.5% of sodium tetraborate, vitellogenin gene transcription was delayed around 2 d compared with control, and began around day 5 after emergence. This result was in agreement with the result of effect of sodium tetraborate on vitellogenin synthesis in which vitellogenin accumulation was delayed when newly emerged flies were treated with 0.5% sodium tetraborate (Fig.7- 4).

Results reported by Handler (1997) and found in this study indicate that vitellogenin gene transcription in the ovary of *A. suspensa* was continuous during oviposition period after it was started at day 3 after emergence, with the highest level after day 5. A similar result was found in *D. melanogaster* in which the yolk protein genes were continuously transcribed from eclosion, as long as the flies were well-fed (Bownes et al., 1988). In the mosquito, *Aedes aegypti*, yolk protein were synthesized in response to a blood meal, vitellogenin gene transcription was switched on after blood feeding, and turned off when the ovary matured until one batch of eggs was produced and next blood feeding was started (Racioppi et al., 1986). Similarly, in *M. domestica*, vitellogenin mRNA was found in the female abdomen from day 2 to day 5 after emergence with highest levels at day 3, and at day 6 to 7 when the ovary approached maturity, vitellogenin gene transcription stopped (Agui et al., 1991).

Handler & Shirk (1988) also showed that the radiolabeling of yolk protein *in vivo* demonstrated the initial presence of low amounts of yolk protein in the ovaries of females 4 to 5 d after emergence, while *in vitro* culture analysis showed yolk protein synthesis in ovaries as early as 3 d. This result indicated that yolk protein gene was not transcribed, or transcribed at very low levels, in the first 2 d after eclosion, which is comparable with our result as described above.

Borovsky (1988) reported in *A. aegypti* that oostatic hormone produced in the ovary, also called trypsin modulating oostatic factor (TMOF), regulated the synthesis of trypsin-like enzyme in midgut, resulting in the regulation of vitellogenin synthesis and egg development.

The results in chapter 5 showed that the activities of proteinases in the midgut were reduced during ovarian development when flies were treated immediately after emergence with 0.5% of sodium tetraborate. Based on this result and data in this study, it appears that reduced proteinase activities would reduce protein digestion, which result in the reduction of yolk protein gene transcription and yolk protein synthesis, or may induce some other factors to regulate indirectively yolk protein gene expression.

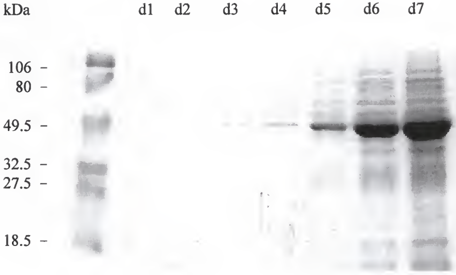


Figure 7-1. Time course of yolk protein accumulation in the ovary of *A. suspensa.*

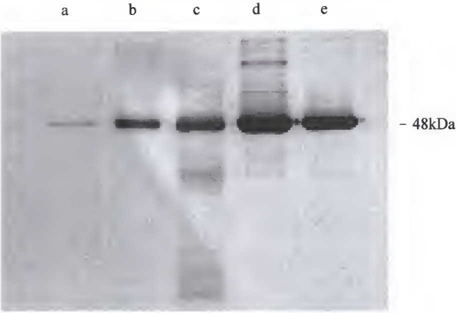


Figure 7-2. Tissue specific yolk protein synthesis viewed by Western blotting.
a: Male abdomen, b: Female fat body, c: Female abdomen, d: Ovary,
e: Eggs. All organs from 7-d-old flies. Each lane loading ½ organ.

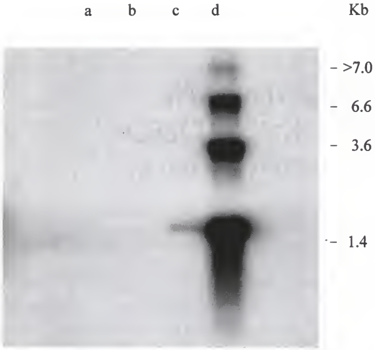


Figure 7-3. Tissue specific yolk protein gene transcription viewed by Northern. blotting, a: 5-d-old male abdomen, b: 5-d-old female fat body, c: 3-d-old female ovary, d: 5-d-old female ovary. Each line loading 1/6 organ.

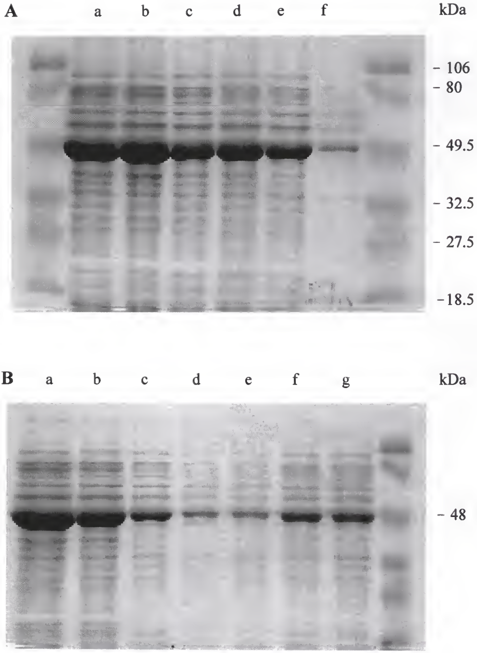


Figure 7-4. Effect of sodium tetraborate on yolk protein accumulation in the ovary of *A. suspensa* treated with different concentrations (A) or treated different sex with 0.5% at different ages (B). A: a: control, b: 0.02%, c: 0.05%, d: 0.1%, e:0.2%, f:0.5%. B: a: control, b: d 0 M, c: d0 F, d: d 0 M&F, e: d 1 M&F, f: d 3 M&F, g: d 5 M&F.

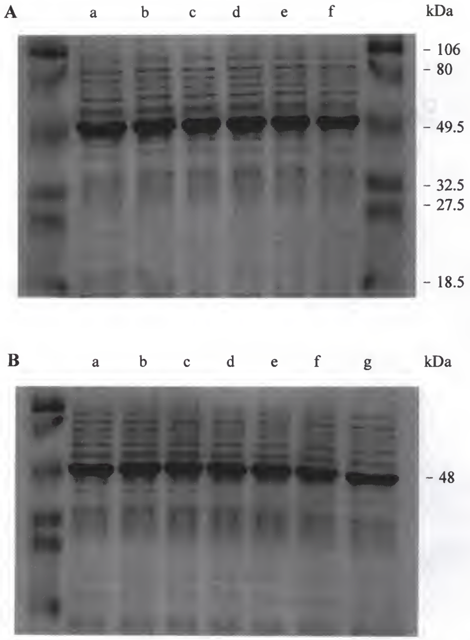


Figure 7-5. Effect of imidacloprid on yolk protein accumulation in the ovary of *A. suspensa* treated with different concentrations (A) or treated different sex with 1.0mg/l at different ages (B). A: a: control, b: 0.05mg/l, c: 0.1mg/l, d:0.2mg/l, e: 0.5mg/l, f:1.0mg/l . B: a: control, b: d 0 M, c: d 0 F, d: d 0 M&F, e: d 1 M&F, f: d 3 M&F, g: d 5 M&F.

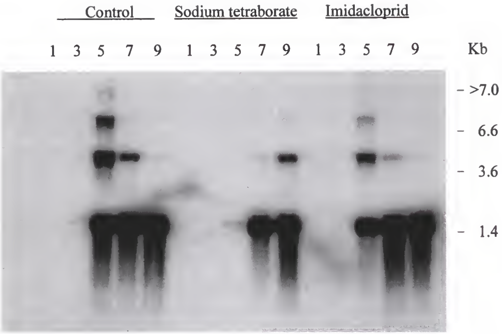


Figure 7-6. Effect of sodium tetraborate (0.5%) and imidacloprid (1.0mg/l) on yolk protein gene transcription of female *A. suspensa*. RNA was extracted from ovaries which were dissected at days 1, 3, 5, 7, and 9 after 24 hr treatment. Each lane loading RNA from 1/6 fly ovary.

CHAPTER 8
SUMMARY AND CONCLUSIONS

The toxicity of sodium tetraborate and imidacloprid to *A. suspensa* was concentration dependent with greater toxicity toward male flies than toward female flies. LC $_{50}$s 48 hr after treatment with sodium tetraborate for male and female *A. suspensa* were significantly different with 2.6% for males and 4.4% for females; with imidacloprid, the difference was not significant. In the 24 hr treatment, mortality with concentrations of 0.02% to 0.5% sodium tetraborate was not different from controls regardless of treated fly age. When flies were treated for 48 hr, 0.5% sodium tetraborate caused 40 -90% mortality for newly emerged flies and 20-60% mortality for 10-d-old flies. If treatment was over 168 hr, more than 95% flies were killed by 0.2% and 0.5% sodium tetraborate within the 168 hr treatment period.

When 10-d-old flies were treated with 0.5% sodium tetraborate by feeding for 24 hr and 48 hr, fecundity was reduced from 15 to 9 and 12 to 6 eggs per female per day; fertility was reduced from 84% to 65% and 87% to 55%, respectively, for 20 days after the treatment ended. The reduction in fecundity and fertility was around 10% higher when flies were treated as newly emerged adults for 24 hr, and oviposition was stopped for 20 d in the 48 hr treatment. If the treatment period was 168 hr, the effective concentration was reduced to 0.1%; 0.2% and 0.5% concentrations killed most flies and stopped the oviposition of survivors for 20 d after the treatment ended. If ≤1.0% sodium tetraborate was mixed into the diet, flies were not repelled for feeding. Imidacloprid had no effect on fecundity and fertility when flies were treated with imidacloprid up to 1.0 mg/l by feeding for 24 hr regardless of fly age. Concentrations of imidacloprid above 1.0mg/l caused 10% or higher mortality and were not tested for fecundity and fertility.

Morphological examination showed that ovarian development of *A. suspensa* was inhibited when flies were treated with 0.5% sodium tetraborate for 24 hr regardless of fly age. The ovary size of 7-d-old flies from the 0.5% treatment was reduced to that of 3- to 4-d-old untreated flies. Light microcopy showed that the yolk protein accumulation in the oocytes of treated flies was reduced. It appears from these data that the inhibition of egg development by sodium tetraborate was due to inhibition of the production or/and the uptake of the yolk protein.

In the midgut of *A. suspensa*, the endoproteinases, trypsin and chymotrypsin, and the exoproteinases, aminopeptidase, carboxypeptidase A and B, were detected . Trypsin and aminopeptidase activities were monitored over the adult life span with higher activities during vitellogenin synthesis, around 4-6 d after emergence. Aminopeptidase and trypsin- and chymotrypsin-like proteinases were also detected in the ovary of *A. suspensa*. Trypsin, aminopeptidase, and azocasein hydrolyzed proteinase activities in the midgut of female *A. suspensa* were reduced around 40-80% for at least 4 d after flies were treated with 0.5% sodium tetraborate for 24 hr. For 1.0mg/l imidacloprid treated flies, trypsin and azocasein hydrolyzed proteinase activities in the midgut were reduced only from day 2 to day 4 after treatment; the reduction on day 4 after treatment was around 20-30% less than in 0.5% sodium tetraborate treated flies. Ovarian proteinase activities in 7-d-old flies were reduced around 80% when flies were treated as newly emerged adults with 0.5% sodium tetraborate by feeding for 24 hr, and 50% with1.0mg/l imidacloprid.

General esterase activity in the abdomen of female *A. suspensa* was significantly increased about 30% on day 4 to day 6 after flies were treated with 0.5% sodium tetraborate. In the male abdomen, general esterase activity was decreased about 30% on day 4 and 40% on day 6 after 24 hr treatment. When flies were treated with imidacloprid ranging from 0.05mg/l to 1.0mg/l for 24 hr, general esterase activity in the abdomen and the whole body of both male and female was not significantly different from control.

Yolk protein synthesis in *A. suspensa* was tissue specific and restricted to the ovary. Yolk protein gene transcription in the ovary began around 3 d after emergence, reached its highest level around day 5, and continued at about the day 5 level to 9 d after emergence. SDS-PAGE revealed that yolk protein synthesis in the ovary was reduced after flies were treated with 0.5% sodium tetraborate; yolk protein accumulation in the 1.0mg/l imidacloprid treated flies was not different from control. Northern blotting showed that yolk protein gene transcription in the ovary of *A. suspensa* was inhibited when flies were treated with 0.5% sodium tetraborate. When flies were treated with 1.0mg/l imidacloprid, the yolk protein mRNA accumulation pattern was not different from control.

The data of this study revealed that the reproduction of *A. suspensa* was affected by inhibiting ovarian development when flies were treated with 0.5% sodium tetraborate by feeding 24 hr. Inhibition of ovarian development appeared to due to the inhibition of yolk protein gene transcription and/or translation. This regulating process may be related to the decrease of midgut proteinase and the increase of esterase activities of female *A. suspensa*, as well as other unknown factors. For 1.0 mg/l imidacloprid treated flies, aminopeptidase activity in the midgut was not affected, only trypsin and azocasein hydrolyzed proteinase activities were reduced from day 2 to 4 after treatment. This reduction might be not enough to affect yolk protein gene expression and inhibit ovarian development of *A. suspensa,* or, general esterase in the female abdomen and other factors may play greater roles in the regulation of yolk protein gene expression.

REFERENCES

Adams, T. S. 1974. The role of juvenile hormone in house fly ovary follicle morphogenesis. J. Insect Physiol. 20: 263-276.

Adams, T. S. & P. A. Filipi. 1983. Vitellin and vitellogenin concentrations during oogenesis in the first gonotrophic cycle of the housefly, *Musca domestica.* J. Insect. Physiol. 29: 723-733.

Adams, T. S. & J. W. Gerst. 1991. The effect of pulse-feeding a protein diet on ovarian maturation, vitellogenin levels, and ecdysteroid titre in housefly, *Musca domestica*, maintained on sucrose. 20: 49-57.

Adams, T. S. & J. W. Gerst. 1992. Interaction between diet and hormones on vitellogenin levels in the housefly, *Musca domestica.* Invert. Reprod. Devel. 21: 91-98.

Adams T. S. & D. R. Nelson. 1990. The influence of diet on ovarian maturation, mating, and pheromone production in the housefly, *Musca domestica*. Invert, Reprod. Devel. 17: 193-201.

Agui, N., T. Shimada, S. Izumi & S. Tomino. 1991. Hormonal control of vitellogenin mRNA levels in the male and female housefly, *Musca domestica.* J. Insect Physiol. 37: 383-390.

Agui, N., M. Takahashi, Y. Wada, S. Izumi & S. Tomino. 1985. The relationship between nutrition, vitellogenin, vitellin and ovarian development in the housefly, *Musca domestica* L. J. Insect Physiol. 31: 715-722.

Ahmad, Z., M. Saleemuddin & M. Siddi. 1980. Purification and characterization of three alkaline proteases from the gut of the larval of army worm, *Spodoptera litura*. Insect Biochem. 10: 667-673.

Albrecht, C. P. & M. Sberman. 1987. Lethal and sublethal effects of avermectin B1 on three fruit fly species (Diptera: Tephritidae). J. Econ. Entomol. 80: 344-347.

Applebaum, S. W. 1985. Biochemistry of digestion. *In* G. A. Kerkut & L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Vol. 4. Pergamon Press, Oxford.

Baker, J. E. 1977. Substrate specificity in the control of digestive enzymes in larvae of the black carpet beetle. J. Insect Physiol. 23: 748-753.

Baker, J. E1981. Resolution and partial characterization of the digestive proteinase from larval of the black carpet beetle, p. 283-315. *In* G. Bhaskaran, S. Friedman & J. G. Rodriguez (eds.), Current Topics in Insect Endocrinology and Nutrition. Plenum, New York.

Baker, J. E1982. Digestive proteinases of *Sitophilus* weevils (Coleptera: Curculionidae) and their responses to inhibitors from wheat and corn flour. Canadian J. Zool. 60: 3206- 3214.

Ballarino, J., M. Ma, T. Ding & C. Lamison. 1990. Development of male-induced ovaries in the gypsy moth, *Lymantria dispar*. Arch. Insect Biochem. Physiol. 16: 221-234.

Baranowski, R., H. Glenn & J. Sivinski. 1993. Biological control of the Caribbean fruit fly (Diptera: Tephritidae). Florida Entomol. 76: 245-251.

Baranowski, R., M., & R. W. Swanson. 1971. The utilization of parachasma cereum (Hymenoptera: Braconidae) as a means of suppressing *Anastrepha suspensa* (Diptera: Tephritidae) populations. Proc. Tall Timbers Conf. On Ecol. Anim. Contr. By Habitat Mange. Feb. 25-27, 249-252.

Bare, O. S. 1945. Boric acid as a stomach poison for the German cockroach. J. Econ. Entomol. 38: 407.

Barillas-Mury, C. V., F. G. Noriega & M. A. Wells. 1995. Early trypsin activity is part of signal transduction system that activates transcription of the late trypsin gene in the midgut of the mosquito, *Aedes aegypti*. Insect Biochem. Molec. Biol. 25: 244-246.

Barrett, A. J. 1994. Classification of peptidases, p. 1-15. *In* A. J. Barrett [ed.], Enzymology. Academic Press, Inc., New York.

Barrett, A. J., A. A. Kembhavi, M. A. Brown, H. Kirschke, C. G. Knight, M. Tamai & K. Hanada. 1982. L-trans-epoxy succinyl-leucylamido(4-guanidino) butane (E-64) and its analogues as inhibitors of cysteine proteinases including cathepsins B, H, and L. Biochem. J. 201: 189-198.

Bell, W. J. 1969. Dual role of juvenile hormone in the control of yolk formation in *Periplaneta americana*. J. Insect. Physiol. 15: 1279-1290.

Benschoter, C. A. 1979. Fumigation of grapefruit with methyl bromide for control of *Anastrepha suspensa*. J. Econ. Entomol. 72: 401-404.

Benschoter, C. A. 1984. Low-temperature storage as a quarantine treatment for the Caribbean fruit fly (Diptera: Tephritidae) in Florida citrus. J. Econ. Entomol. 77: 1233-1235.

Benschoter, C. A. 1988. Methyl bromide fumigation and cold storage as treatment for California stone fruits and pears infested with the Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 81: 1665-1667.

Bigley, W. S. & F. W. Plapp. 1960. Cholinesterase and aliesterase activity in organic phosphorus-susceptible and –resistant house flies. J. Econ. Entomol. 53: 360-364.

Boake, C. R. B. & S. Moore. 1996. Male acceleration of ovarian development in *Drosophila silvestris* (Diptera: Drosophidae): What is the stimulus? J. Insect Physiol. 42: 649-655.

Boiteau, G., W. P. L. Osborn & M. E. Drew. 1997. Residual activity of imidacloprid controlling Colorado potato beetle (Coleoptera: Chrysomelidae) and three species of potato colonizing aphids (Homoptera: Aphidae). J. Eco. Entomol. 90: 309-319.

Bond, J. S. & P. E. Butler. 1987. Intracellular proteinases. Ann. Rev. Biochem. 56: 333-364.

Bonning, B. C., W. Loher & B. D. Hammock. 1997. Recombinant juvenile hormone esterase as a biochemical anti-juvenile hormone agent: effect on ovarian development in *Acheta domesticus*. Arch. Insect Biochem. Physiol. 34: 359-368.

Borkovec, A. B., J. A. Settepani, G. C. Labrecque & R. L. Fye. 1969. Boron compounds as chemosterilants for house flies. J. Econ. Entomol. 62: 1472-1480.

Borovsky, D. 1982. Release of the egg development neurosecretory hormone-release factor from ovaries of mosquitoes fed blood. J. Insect Physiol. 28: 503-508.

Borovsky, D. 1985. Characterization of proteolytic enzymes of the midgut and excreta of the biting *fly Stomoxys calcitrans*. Arch. Insect Biochem. Physiol. 2: 145-159.

Borovsky, D. 1988. Oostatic hormone inhibits biosynthesis of midgut proteolytic enzymes and egg development in mosquito. Arch. Insect Biochem. Physiol. 7: 187-210.

Borovsky, D. & P. L. Whitney. 1987. Biosynthesis, purification, and characterization of *Aedes aegypti* vitellin and vitellogenin. Arch. Insect Biochem. Physiol. 4: 81-99.

Bownes, M. 1979. Three genes for three yolk proteins in *Drosophila melanogaster*. FEBS Letters. 100: 95-98.

Bownes, M. 1989. The roles of juvenile hormone, ecdysone and ovary in the control of *Drosophila* vitellogenesis. J. Insect Physiol. 35: 409-413.

Bownes, M. & M. Blair. 1986. The effects of a sugar diet and hormones on the expression of the *Drosophila* yolk-protein genes. J. Insect Physiol. 32: 493-501.

Bownes, M. & B. D. Hames. 1977. Accumulation and degradation of the three yolk proteins in *Drosophila melanogaster*. J. Exp. Zool. 200: 149-156.

Bownes, M. & B. D. Hames. 1978. Analysis of the yolk protein in *Drosophila melanogaster*: translation in a cell free system and peptide analysis. FEBS Letter. 96: 327-331.

Bownes, M. & L. Partridge. 1987. Transfer of molecules from ejaculate to females in *Drosophila melanogaster* and *Drosophila pseudoobscura*. J. Insect Physiol. 33: 941-947.

Bownes M. & S. Reid. 1990. The role of the ovary and nutritional signals in the regulation of body yolk protein gene expression in *Drosophila melanogaster*. J. Insect Physiol. 36: 471-479.

Bownes, M., A. Scott & M. Blair. 1987. The use of an inhibitor of protein synthesis to investigate the roles of ecdysteroids and sex-determination genes on the expression of the gene encoding the *Drosophila* yolk proteins. Development. 101: 931-941.

Bownes, M., A. Scott & A. Sherras. 1988. Dietary components modulate yolk protein gene transcription in *Drosophila melanogaster*. Development. 103: 119-128.

Braga, M. A. S. & F. S. Zucoloto. 1981. Estudos sobre a melhor concentracao de amino-acids paramoscas adultas *Anastrepha oblique* (Diptera: Tephitidae). Revta. Brasil. Biol. 41: 75-79.

Brennan, M. D., A. J. Weiner, T. J. Goralski & A. P. Mahowald. 1982. The folllicle cells are a major site of vitellogenin synthesis in *Drosophila melanogaster*. Develop. Biol. 89: 225-236.

Briegel, H. 1975. Excretion of proteolytic enzymes by *Aedes aegypti* after a blood meal. J. Insect Physiol. 21: 1681-1684.

Briegel, H. & A. O. Lea. 1975. Relationship between protein and proteolytic activity in the midgut of mosquitoes. J. Insect Physiol. 21: 1597-1604.

Broadway, R. M. 1989. Characterization and ecological implications of midgut proteolytic activity in larval *Pieris rapae* and *Trichoplusia ni*. J. Chem. Ecol. 15: 2101-2113.

Brown, M. R., A. S. Raikhel & A. O. Lea. 1985. Ultrastructure of midgut endocrine cells in the adult mosquito *Aedes aegypti* (Diptera). Tissue Cell. 17: 709-721.

Budia, F & E. Vinuela. 1996. Effect of cyromazine on adult *C. capitata* (Diptera: Tehritidae) on mortality and reproduction. J. Econ. Entomol. 84: 826-831.

Buning, J. 1997. The insect ovary, ultrastructure. Previtellogenic growth and evolution. Chapman & Hall, London, New York, pp. 425.

Burditt, A. K. Jr. 1982. *Anastrepha suspensa* (Loew) (Diptera: Tephitidae) McPhail traps for survey and detection. Florida Entomol. 65: 367-373.

Burditt, A. K. Jr., F. Lopez-D, L. F. Steiner, D. L. Von Windeguth, R. Baranowski & M. Anwar. 1974. Application of sterilization techniques to *Anastrephe suspensa* (Loew) in Florida, USA. Sterility principles for insect control. IAEA-SM-186/4Z. PP. 93-101.

Burditt, A. K. Jr. & D. L. Von Windeguth. 1975. Semi-trailer fumigation of Florida grapefruit infested with larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Leow). Proc. Florida State Hort. Soc. 88: 318-323

Burk, T. 1983. Behavioral ecology of mating in the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephitidae). Florida Entomol. 66: 330-343.

Calkins, C. O. 1993. Future directions in control of the Caribbean fruit fly (Diptera: Tephitidae). Fla. Entomol. 76: 263-269.

Calkins, C. O., K. A. A. Draz & B. J. Smittle. 1988. Irradiation/sterilization techniques for *Anastrepha suspensa* Loew and their impact on behavioral quality. *In* Modern Insect Control: Nuclear Techniques and Biotechnology. International Atomic Energy Agency, Vienna.

Champlain, R. A. & F. W. Fisk. 1956. The digestive systems of the stable fly, *Stomoxys calcitrans* (L.). Ohio. J. Sci. 56: 52-62.

Chang, F., C. L. Hsu & L. Jurd. 1988. Effect of benzyl-1,3-benzodioxole analogs on reproduction in the mediterranean fruit fly, *Ceretitis capitata* (Wiedemann). Insect Sci. Applic. 9: 381-388.

Chang, F., E. B. Jang, C. Hsu & L. Jurd. 1991. Effect of benzodioxole-1, 3, -benzodioxole derivatives on juvenile hormone binding by components of the hemolymph from the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann. Comp. Biochem. Physiol. 99C: 15-20.

Chang, F., E. B. Jang, C. Hsu & L. Jurd. 1994. Effect of benzodioxole-1, 3, -benzodioxole derivatives and their effects on the reproductive physiology of insect. Arch. Insect Biochem.Physiol. 27: 39-51.

Chang, F., E. B. Jang, C. Hsu, M. C. Ma & L. Jurd. 1994. Benzodioxole-1, 3, -benzodioxole derivatives on juvenile hormone binding by components of the hemolymph from the Mediterranean fruit fly, *Ceretitis capitata* Wiedemann.Comp. Biochem. Physiol. 99: 15-22.

Chang, S. C. 1979. Laboratory evaluation of diflubenzuron, penfluron, and Bay sir 8514 as female sterilants against the house fly. J. Econ. Entomol. 72: 479-481.

Chen, A. C., H. R. Kim, R. T. Mayer & J. O. Norman. 1987. Vitellogenesis in the stablefly, *Stomoxys calcitrans*. Comp. Biochem. Physiol. 88B: 897-903.

Chen, D. S. 1984. The functional morphology and biochemistry of insect male accessory glands and their secretions. Ann. Rev. Entomol. 29: 233-255.

Chen, T. T., P. Couble, R. Abu-Hakima & G. R. Wyatt. 1978. Juvenile hormone-controlled vitellogenin synthesis in *Locusta migratoria* fat body. Hormonal induction in vivo. Develop. Biol. 69: 70-72.

Chen, T. T., P. W. Strahlendor & G. R. Wyatt. 1978. Vitellogenin and vitellin from Locusts (*Locusta migratioria*). J. Biol. Chem. 253: 5325-5331.

Chia, W. K. & P. E. Morrison. 1972. Autoradiographic & ultrastructure studies on the original of yolk proteins in the housefly, *Musca domestica* L. Canndian J. Zool. 50: 1569-1582.

Chinzei, Y., H. Chino & G. R. Wyatt. 1981. Purification and properties of vitellogenin and vitellin from *Locusta migratoria*. Insect Biochem. 11: 1-7.

Cho, W. L., K. W. Deitsh & A. S. Raikhel. 1991. An extra-varian protein accumulated in mosquito oocytes is a carboxypeptidase activited in embryos. Proc. Natl. Acad.Sci. USA. 88: 10821-10824.

Christeller, J. T., W. A. Laing, N. P. Markwick & E. P. J. Burgess. 1992. Midgut protease activities in 12 phytophagous lepidopteran larvae: dietary and protease inhibitor interactions. Insect Biochem. Mol. Biol. 22: 735-746.

Christeller, J. T., W. A. Laing, B. D. Shaw & E. P. J. Burgess. 1990. Characterization and partial purification of the digestive proteases of the black field cricket *Teleogryllus commodus* (Walker): elastase is a major component. Insect Biochem. 20: 157-164.

Christeller, J. T., B. D. Shaw, S. E. Gardiner & J. Dymock. 1989. Partial purification and characterization of the major midgut proteases of grass grub larvae (*Costelytrazealandica* Coleoptera: Scarabaeidae). Insect Biochem. 19: 221-231.

Christopher, M. S. M. & S. Mathavan. 1985. Regulation of digestive enzyme activity in the larvae of catopsilia crocale (lepidoptera). J. Insect Physiol. 31: 217-221.

Chon, T. S., D. Ling & C. Schal. 1990. Effects of mating and grouping on oocyte development and pheromone release activities in *Supella longipalpa* (Dictyoptera: Blatellidae). Environ. Entomol. 19: 1716-1721.

Cochran, D. G. 1985. Mortality and reproductive effects of avermectin B1 fed to German cockroaches. Entomol. Exp. Appl. 37: 83-88.

Cochran, D. G. 1995. Toxic effects of boric acid on the German cockroach. Experientia 51: 561-563.

Craddock, E. M. & C. R. B. Boake. 1992. Onset of vitellogenesis in female *Drosophila silvestris* is accelerated in the presence of sexually mature males. J. Insect Physiol. 38;643-650.

Cusson, M. & J. Delisle. 1996. Effect of mating on plasma juvenile hormone esteraseactivity in female of *Choristoneura fumiferana* and *C. rosaceana*. Arch. Insect Biochem. Physiol. 32: 585-599.

Cymborowski, B. 1992. Insect Endocrinology, Chap. 3, and Chap. 5.Elsevier, Pwn-Polish Scientific Publishers, Amsterdam, Oxford, New York, Tokyo.

Dadd, R. H. 1985. Nutrition: Organism, p. 313-389. *In* G. A. Kerkut & I. L. Gilbert (eds), Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Pergamon Press, Oxford.

Dahlmann, B., K. D. Jany & G. Pfleiderer. 1978. The midgut endopeptidases of the honeybee (*Apis mellifica*): comparison of the enzymes in different ontogenetic stages. Insect Biochem. 8: 203-211.

Dary, O., G. P. Georghiou, E. Parsons & N. Pasteur. 1990. Microplate adaptation of Gomor's assay for quantitative determination of general esterase activity in single insect. J. Econ. Entomol. 83: 2187-2192.

De Bianchi, A. G., M. Coutinho, S. D. Pereira, O. Marinotti & H. J. Targa. 1985a. Vitellogenin and vitellin of *Musca domestica*, quantification and synthesis by fat bodies and ovaries. Insect Biochem. 15: 77-84.

De Bianchi, A. G., M. Coutinho, S. D. Pereira, O. Marinotti & H. J. Targa. 1985b. Vitellogenin and vitellin of *Musca domestica*. Insect Biochem. 15: 173-180.

De Cock, A., P. Declercq, L. Tirry & D. Degheele. 1996. Toxicity of diafenthiuron and imidacloprid to the predatory bug *Podisus maculiventris* (Heteroptera: Pentaomidae). Environ. Entomol. 25: 476-480.

De Kort, C. A. D. & N. A. Granger. 1996. Regulation of JH titers: The relevance of degradative enzymes and binding proteins. Arch. Insect Biochem. Physiol. 33: 1-26.

Denlinger, D. L. 1971. Autogeny in the flesh fly *Sarcophaga argyrostoma*. Ann. Entomol. Soc. America. 64: 961-962.

Diaz, F., J. Toledo, W. Enkerlin & J. Hernandez. 1996. Cyromazine: effects on three species of *Anatrepha*. Fruit fly pest: a world assessment of their biology and mangement. 333-337.

Di Ilio, V., M. Cristofaro, D. Marchini, P. Nobili & R. Dallai. 1999. Effects of a neem Compound on the fecundity and longevity of *Ceratitis capitata* (Diptera: Tephritidae) J. Econ. Entomol. 92: 76-82.

Dong, Y-J., E. B. Jang, L-L. Cheng, C-C. Chen & R. F. Hou. 1997. Effect of topsoil treated with J3230 (5-propyl-1-en-3-oxy-6(1-(4-methyoxyphenyl)ethyl)-1,3-benzodioxole) on reproduction of the Oriental fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 90: 535-539.

Downe, A. E. R. 1975. Internal regulation of rate of digestion of blood meals in the mosquito, *Aedes aegypti*. J. Insect Physiol. 21: 1835-1839.

Eguchi, M., S. Furukawa & A. Iwamoto. 1972. Proteolytic enzyme in the midgut of the Pharate adult of the silkworm, *Bombyx mori*. J. Insect Physiol. 18: 2457-2467.

Engelmann, F. 1969a. Female specific protein: biosynthesis controlled by corpus allatum in *Leucophaca maderas*. Science. 165: 407-409.

Engelmann, F. 1969b. Food-stimulated synthesis of intestinal proteolytic enzymes in the cockroch *Leucophaea maderae*. J. Insect. Physiol. 15: 217-235.

Engelmann, F. 1979. Insect vitellogenin: identification, biosynthesis, and the role in vitellogenesis. Adv. Insect Physiol. 14: 49-108.

Ferenz, H. J., E. W. Lubzens & H. Glass. 1981. Vitellin and vitellogenin incorporation by isolated oocytes of *Locusta migratoria migratorioides* (R. and F.). J. Insect Physiol. 27: 869-875.

Ferro, M. I. T & F. S. Zucoloto. 1990. Effect of the quantity of dietary amino acids on egg production and laying by *Ceratitis capitata*. Brazilian J. Med. Biol. Res. 23: 525-531.

Fourney, R. M., G. F. Pratt, D. G. Harnish, G. R. Wyatt & B. N. White. 1982. structure and synthesis of vitellogenin and vitellin from *Calliphora erythrocephala*. Insect Biochem. 12: 311-321.

Friedel, T. & C. Gillott. 1976. Extraglandular synthesis of accessory reproductive gland components in male *Melanoplus sanguinipes*. J. Insect. Physiol. 22: 1309-1314.

Friedel T. & P. A. McDonell. 1985. Cyromazine inhibits reproduction and larval development of the Australian sheep blow fly (Dip: Calliphoridae). J. Econ. Entomol. 78: 868-873.

Galun, R., S. Gothilf & S. Blondheim. 1981. Protein and sugar hunger in the Mediterranean fruit fly: *Ceratitis capitata*. 8-12, Proc. 5th European Chemoreception Res. Org. Symposium.

Gellissen, G., F. Waje, E. Cohen, H. Emmerich, S. W. Applebaum & J. Flossdorf. 1976. Purification and properties of oocyte vitellin from migratory locust. J. Comp. Physiol. 108B: 287-301.

Gemmill, R. M., M. Hamblin, R. L. Glaser, J. V. Racioppi, J. L. Marx, B. N. White, J. M. Calvo, M. F. Wolfner & H. H. Hagedorn. 1986. Isolation of mosquito vitellogenin genes and induction of expression by 20-hydroxyecdysone. Insect Biochem. 16: 761- 774.

Giebel, W., R. Zwilling & G. Peleiderer. 1971. The evolution of endopeptidases XII. The proteolytic enzymes of the honebee (*Apis mellifica* L.) Comp. Biochem. Physiol. 38B: 197-210

Gilbert, D. G. 1981. Ejaculate esterase 6 and initial sperm use by female *Drosophila melanogaster*. J. Insect Physiol. 27: 641-650.

Gilbert, D. G. & R. C. Richmond. 1982. Esterase 6 in *Drosophila malanogaster*: reproduction function of active and null males at low temperature. Proc. Natl. Acad. Sci. USA. 79: 2962-2966.

Gilbert, D. G., R. C. Richmond & K. B. Sheehan. 1981a. Studies of esterase 6 in *Drosophila melanogaster* V. progeny production and sperm use in females inseminated by males caring active or null alleles. Evolution. 35: 21-37.

Gilbert, D. G., R. C. Richmond & K. B. Sheehan. 1981b. Studies of esterase 6 in *Drosophila melanogaster* VII. The timing of remating in females inseminated by males having active or null alleles. Behav. Gen. 11: 195-208.

Gooding, R. H. 1966. In vitro properties of proteinases in the midgut of *Aedes aegypt* L. and *Culex fatigans* (Wiedemann). Comp. Biochem. Physiol. 17: 115-121.

Gooding, R. H. 1973. The digestive processes of haematophagous insects IV. Secretion of trypsin by *Aedes aegypti* (Diptera: Culicidae). Canadian Entomol. 105: 599-603.

Gooding, R. H. 1974. Digestive Processes of haematophagous insects: Control of trypsin secretion in *Glossina marsitans*. J. Insect Physiol. 20: 957-964.

Gooding, R. H. 1977. Digestive processes of haematophagous insects XII. Secretion of trypsin and carboxypeptidase B by *Glossina morsitans* Westw (Diptera: Glossinidae). Canadian J. Zool. 55: 215-222.

Gooding, R. H. & L. T. Huang. 1969. Trypsin and chymotrypsin from the beetle *Pterostichus melanarius*. J. Insect Physiol. 15: 325-339.

Gould, W. P. 1988. A hot water/cold storage quarantine treatment for grapefruit infested with the Caribbean fruit fly. Proc. Florida State Hort. Soc. 101: 190-192.

Grace, J. K. 1991. Response of Eastern and Formosan subterranean termites (Isoptera: Rhinoterminidae) to borate dust and soil treatments. J. Econ. Entomol. 84: 1753-1757.

Graf, G. & H. Briegel. 1985. Isolation of trypsin isoenzymes from the mosquito *Aedes aegypti* (L.). Insect Biochem. 15: 11-618.

Graf, G. & H. Briegel. 1989. The synthesis pathway of trypsin in the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae) and in vitro stimulation in isolated midgut. Insect Biochem. 19: 129-137.

Greany, P. D. & C. Riherd. 1993. Caribbean fruit fly status, economic importance, and control (Diptera: Tephritidae). Florida Entomol. 76: 209-211.

Greany P. D., P. E. Shaw, P. L. Davis & T. T. Hatton. 1985. Senescence-related susceptibility of Marsh grapefruit to laboratory infestation by *Anastrepha suspensa* (Diptera: tephitidae). Florida Entomol. 68: 144-150.

Greenberg, B. & D. Paretsky. 1955. Proteolytic enzymes in the house fly, *Musca Domestica* (L.). Ann. Entomol. Soc. America. 48: 46-50.

Gwynne, D. G. 1984. Courtship feeding increases female reproductive success in bush crickets. Nature. 307: 361-363..

Hagedorn, H. H. 1983. The role of ecdysteroids in the adult insect, p. 271-304. *In* R. G. H. Downer & H. Laufer (eds.), Endocrinology of Insects, Alan R. Liss.

Hagedorn, H. H. 1985. The role of ecdysteroids in the adult insect, p. 205-262. *In* G. A. Kerkut & L. I. Gilbert (eds.), Comprehensive Insect Physiology Biochemistry and Phamacology. Pergamon Press, Oxford.

Hagedorn, H. H. & C. L. Judson. 1972. Purification and site of synthesis of *Aedes aegypti* yolk protein. J. Exp. Zool. 182: 367-377.

Hagedorn, H. H.& J. G. Kunkel. 1979. Vitellogenin and vitellin in insects. Ann. Rev. Entomol. 24: 475-505.

Hamed, M. B. B. & J. Attias. 1987. Isolation and partial characterization of two alkline proteases of the greater wax moth *Galleria mellonella* (L.). Insect. Biochem. 17: 653-658.

Hammock, B. D. 1985. Regulation of the juvenile hormone titer: degradation, p. 431-472. *In* G. A. Kerkut & L. I. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Vol.7. Pergamon Press, Oxford.

Hanaoka, K. & H. H. Hagedorn. 1980. Brain hormone control of ecdysone secretion by the ovary in a mosquito, p. 467-480. *In* J. A. Hoffmann [ed.], Progress in Ecdyson Research, Elsevier/North-Holland Press, Amsterdam.

Handler, A. M. 1997. Developmental regulation of yolk protein gene expression in *Anastrepha suspensa*. Arch. Insect Biochem. Physiol. 36: 25-35.

Handler, A. M. & J. H. Postlethwait. 1978. Regulation of vitellogenin synthesis in *Drosphila* by ecdysterone and juvenile hormone. J. Exp. Zool. 206: 247-254.

Handler, A. M. & P. D. Shirk. 1986. Analysis of yolk proteins from the Caribbean fruit fly, *Anastrepha suspenusa*. *In* A. P. Economopoulos [ed.], Fruit Fly, Proceeding of the Second International Symposium, Elsevier Science Publishing Co. Inc., New York.

Handler, A. M. & P. D. Shirk. 1988. Identification and analysis of the major yolk polypeptide from the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Arch. Insect Biochem. Physiol. 9: 91-106.

Hanzlik, T. N. & B. D. Hammock. 1987. Characterization of affinity purified juvenile hormone esterase from *Trichoplusia ni.* J. Biol. Chem. 13584-13591.

Hardie, J. 1995. Hormones and reproduction. *In* S. R. Leather & J. Hardie (eds.), Insect Reproduction. CRC Press Inc., Boca Raton, FL.

Harnish, A. M. & B. N. White. 1982. Insect vitellins: identification, purification, and Characterization from eight orders. J. Exp. Zool. 220: 1-10.

Hartly, B. S. 1960. Proteolytic enzymes. Ann. Rev. Biochem. 29: 45-72.

Haynes, J. W., W. l. Mcgovern & J. E. Wright. 1981. Diflubenzuron (solvent-water suspension) dip for boll weevils: Effects measured by flight, sterility, and sperm transfer. Environ. Entomol. 10: 492-495.

Healy, M. J., M. M. Dumancic & J. G. Oakeshott. 1991. Biochemical and physiological studies of soluble esterase from *Drosophila melanogaster*. Biochem. Genet. 29: 365-388.

Heath, R. R, N. D. Epsky, P. J. Landolt & J. Sivinski. 1993. Development of attractants for monitoring Caribbean fruit fly (Diptera: Tephritidae). Florida Entomol. 76: 233-243.

Herndon, L. A. & M. F. Wolfner. 1995. A *Drosophila* seminal fluid protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. Proc. Natl. Acad. Sci. USA. 92: 10114-10118.

Hiremath, S. & S. Eshita. 1992. Purification and characterization of vitellogenin from the Gypsy moth, *Lymantria dispar*. Insect biochem. Molec. Biol. 22: 605-611.

Ho, S. H. & K. I. Sudderuddin. 1976. An in vitro study of the non-specific esterases of the melon fly, *Dacas cacurbitae* Coq. and their reactions with organophosphate and carbamate compounds. Comp. Biochem. Physiol. 54C: 95-97.

Hoffmann, K. H. 1995. Oogenesis and the females reproductive system. *In* S. R. Leather & J. Hardie (ed.), Insect Reproduction. CRC Press Inc., Boca Raton, FL.

Hogsette, J. A. & P. G. Koehler. 1992. Toxicity of Aqueous solutions of boric acid and polybor 3 to house flies (Diptera: Muscidae). J. Econ. Entomol. 85: 1209-1212.

Hogsette, J. A. & P. G. Koehler. 1994. Repellency of Aqueous solutions of boric acid and polybor 3 to house flies (Diptera: Muscidae). J. Econ. Entomol. 87: 1033-1037.

Holler, T. C. & D. L. Harris. 1993. Effect of sterile release of Caribbean fruit flies (Diptera: Tephritidae) against wild population in urban hosts adjacent to commercial citrus. Florida Entomol. 72: 251-257.

Hori, K., R. Atalay & S. Araki. 1981. Digestive enzymes in the gut and salivary gland of the adult horn fly *Haematobia irritans* (Diptera: Muscidae). Appl. Entomol. Zool. 16-16-23.

House, H. L. 1974. Digestion, p. 63-117. *In* M. Rockstein (ed.), Physiology of Insecta, Vol 5. Academic, New York.

Houseman, J. G. 1978. A thiol-activited digestive proteinase from adults of *Rhodnius prolixus* Stal (Hemiptera: Reduviidae). Canadian J. Zool. 56: 1140-1143.

Houseman, J. G. & A. E. R. Downe. 1980. Endoproteinase activity in the posterior midgut of *Rhodnius prolixus* Stal (Hemiptera: Reduviidae). Insect Biochem. 10: 363-366.

Houseman, J. G. & A. E. R. Downe. 1981. Exoproteinase activity in the posterior midgut of *Rhodnius prolixus* Stal (Hemiptera: Reduviidae). Insect Biochem. 11: 579-582.

Houseman, J. G. & A. E. R. Downe. 1983. Activity cycles and the control of four digestive proteinases in the posterior midgut of *Rhodnius prolixus* Stal (Hemiptera: Reduviidae). J. Insect Physiol. 29: 141-148.

Houseman, J. G., A. E. R. Downe & B. J. R. Philogene. 1989. Partial characterization of proteinase activity in the larval midgut of the European corn borer *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae). Canadian J. Zool. 67: 864-868.

Houseman, J. G., A. M. Larocque & N. M. R. Thie. 1991. Insect proteinases, plant proteinase inhibitors, and possible pest control. Mem. Entomol. Soc. Canada. 159: 3-11.

Houseman, J. G. & P. E. Morrison. 1986. Absence of female-specific protein in the Hemolymph of stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Arch. Insect Biochem. Physiol. 3: 205-213.

Houseman, J. G., P. E. Morrison & A. E. R. Down. 1985. Cathepsin B and aminopeptidase activity in the posterior midgut of *Phymata wolffi* Stal (Hemiptera: Phymitadae). Canadian J. Zool. 63: 1288-1291.

Houseman, J. G. & N. M. R. Thie. 1993. Difference in digestive proteolysis in the stored maize beetles: *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Coleoptera: Bostrichidae). J. Econ. Entomol. 86: 1049-1054.

Hsu, C., F. Chang, H. F. Mower, L. J. Groves & L. Jurd. 1989. Effect of orally administered 5-ethoxy-6-(4-methoxyphenyl) methyl-1,3-benzodioxole on reproduction of the Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol.82: 1046-1053.

Hsu, C., F. Chang, H. F. Mower & L. Jurd. 1990. Effect of topsoil treated with J2581 on reproduction in the Oriental fruit fly (Diptera: Tephritidae). J. Econ. Entomol.83: 1261-1266.

Hummel, B. C. W. 1959. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Canadian J. Biochem. and Physiol. 37: 1393-1399.

Huybrechts, G. & A. De Loof. 1977. Induction of vitellogenin synthesis in male *Sarcophaga bullata* by ecdysterone. J. Insect Physiol. 23: 1359-1362.

Huybrechts, G. & A. De Loof. 1981. Effect of ecdysterone on vitellogenin concentration in hemolymph of male and female *Sarcophaga bullata*. Int. J. Invert. Reprod. 3: 157-168.

Ikeda, M., T. Sasaki & O. Yamashita.1990. Purification and characterization of protease responsible for vitellin degradation of the silkworm, *Bombyx mori*. Insect Biochem. 20: 725-734.

Indrasith, L. S., T. Sasaki & O. Yamashita. 1988. An unique protease responsible for selective degradation of a yolk protein in *Bombyx mori*. J. Biol. Chem. 263: 1045-1051.

Izumi, S., S. Tomino & H. Chino. 1980. Purification and molecular properties of vitellin From the silkworm, *Bombyx mori*. Insect Biochem. 10: 199-208.

Izumi, S., K. Yano & S. Y. Takahash. 1994. Yolk proteins from insect eggs: structure, biosynthesis and programmed degradation during embryogenesis. J. Insect Physiol. 40: 735-746.

Jesudason, P., K. Venkatesh & R. M. Roe. 1990. Haemolymph juvenile hormone Esterase during the life cycle of the tobacco hornworm, *Manduca sexta* (L.). Inesct Biochem. 20: 593-604.

Johnston, K. A., M. J. Lee, C. Brough, V. A. Hilder, A. M. R. Gatehouse & J. A. Gatehouse. 1995. Protease activities in the larval midgut of *Heliothis virescens*: evidence for trypsin and chymotrypsin-like enzymes. Insect. Biochem. Molec. Biol. 25: 375-383.

Johnston, K. A., M. J. Lee, J. A. Gatehouse & J. H. Anstee. 1991. The partial purification and characterization of serine protease activity in midgut of larval *Helicoverpa nubilalis*. Insect. Biochem. 21: 389-397.

Jones, G., K. D. Wing, D. Jones & B. D. Hammock. 1981. The source and action of head factors regulating juvenile hormone esterase in larvae of the cabbage looper, *Trichoplusia ni*. J. Insect Physiol. 27: 85-91.

Kageyama, T. & S. Y. Takahashi. 1990. Purification and characterization of a cystine protease from silkworm eggs. European J. Biochem. 193: 203-210.

Kageyama, T., S. Y. Takahashi & K. Takahashi. 1981. Occurrence of thiol proteinases in the eggs of the silkworm, *Bombyx mori*. J. Biochem. 90: 665-671.

Kawamura, M., A. Wadano & K. Miura. 1987. Purification and characterization of insect cathepsin D. Insect Biochem. 17: 77-83.

Kelly, T. J., M. S. Fuchs & S. H. Kang. 1981. Induction of ovarian development in autogenous *Aedes atropalpus* by juvenile hormone and 20-hydroxyecdyson. Int. J. Invert. Reprod. 3: 101-112.

Kindle, H., R. Konig & B. Lanzrein. 1988. *In vitro* uptake of vitellogenin by follicles of the cockroach *Nauphoeta cinerea*: comparison of artificial media with hemolymph media and the role of vitellogenin concentration and juvenile hormone. J. Insect Physiol. 34: 541-548.

King, R. C. 1970. Ovarian development in *Drosophila melanogaster*, Academic press, New York and London.

Kitch, L. W. & L. L. Murdock. 1986. Partial characterization of a major gut thiol proteinase from larvae of *Callosobruchus maculatus* F. Arch. Insect Biochem. Physiol. 3: 561-575.

Klotz, J. H., K. M. Vail & D. F. Williams. 1997. Toxicity of boric acid-sucrose water bait to *Solenopsis invicta* (Hymenoptera: Formicidae). J. Econ. Entomol. 90: 488-491.

Knecht, M., H. E. Hagenmaier & E. Zebe. 1974. The proteases in the locust *Locusta migratoria*. J. Insect Physiol. 20: 461-470.

Koopmanschap, A. B. & C. A. D. De Kort. 1989. Carboxyesterases of high molecular Weight in the hemolymph of *Locusta migratoria*. Experientia. 45: 327-330.

Kotze, A. C. 1992. Effects of cyromazine on reproduction and offspring development in *Lucilia cupria* (Diptera: Calliphoridae). J. Econ. Entomol. 85: 1614-1617.

Kraut, J. 1977. Seine protease: structure and mechanism of catalysis. Ann. Rev. Biochem. 46: 331-358.

Kubli, E. 1996. The *Drosophila* sex-peptide: a peptide pheromone involved in reproduction, p. 99-128. *In* P. Wassarman [ed.], Advances in Developmental Biochemistry. JAI Press, New York.

Kuk-Meiri, S., N. Lichtenstein, A. Shulo & M. P. Pener. 1966. Cathepsin-type proreolytic activity in the developing eggs of the African migratory locust (*Locusta migratoria migratorioides* R. and F.). Comp. Biochem. Physiol. 18: 783-795.

Kulakosky, P. C. & W. H. Telfer. 1989. Kinetics of yolk precursor uptake in *Hyalophora ceropia*: stimulation of microvitellin endocytosis by vitellogenin. Insect Biochem. 19: 336-343.

Kunkel, J. G. & J. H. Nordin. 1985. Yolk proteins. *In* G. A. Kerkut & L. I. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Vol. 1, Chap. 4. Pergamon, Oxford.

Kunz, P. A. 1978. Resolution and properties of the proteinases in adult *Aedes aegypti* (L.). Insect Biochem. 8: 169-175.

Kuribayashi, S. 1981. Studies on the effect of pesticides on the reproduction of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) II. Ovicidal action of organophosphorus insecticides administered during the larval stage. Appl. Entomol. Zool. 16: 423-431.

Lamy, M. 1984. Vitellogenesis, Vitellogenin and Vitellin in the males of insects. A Review. Inter. J. Invert. Reprod. Develop 7: 311-325.

Lang, J. T. & R. E. Treece. 1972. Boric acid effects on face fly fecundity. J. Econ. Entomol. 65: 740-741.

Langley, P. A., M. A. Trewern & L. Jurd. 1982. Sterilising effects of benzyl-1,3,-benzodioxoles on the testse fly *Glossina morsitans* (Westwood) (Diptera: Glossinidae). Bull Entomol. Res. 72: 473-477.

Laposata, M. M. & W. A. Dunson. 1998. Effects of boron and nitrate on hatching success of amphibian eggs. Arch. Environ. Contam. Toxicol. 35: 615-619.

Lawrence, P. O. 1983. Age-specific fecundity and offspring survivoship in the caribbean fruit fly *Anastrepha suspensa* after diflubenzuron treatment. Insect Sci. Appl. 4: 285-290.

Lawrence, P. O. 1989. Differential mortality, fecundity, and egg viability of Caribbean fruit fly, *Anastrepha suspensa* adults fed on three diets. Insect Sci. Appli. 10: 353-357.

Lea, A. O. 1967. The medial neurosecretory cells and egg maturation in mosquitoes. J. Insect. Physiol. 13: 419-523.

Lea, A. O. 1972. Endocrine of nutritional requirements for insemination and vitellogenesis in *Musca domestica*. American Zool. 12: 661-667.

Lea, A. O. & E. Van Handel. 1982. A neurosecretory hormone-release factor from ovaries of mosquitoes fed blood. J. Insect Physiol. 28: 503-508.

Leahy, M. G. & G. B. Craig Jr., 1965. Male accessory gland substance as a stimulatant for oviposition in *Aedes aegypti* and *A. albopictus*. Mosq. News. 25;448-452.

Lee, M. J. & J. H. Anstee. 1995. Endoproteases from the midgut of larval *Spodoptera littoralis* include a chymotrypsin-like enzyme with an axtended binding site. Insect Biochem. Molec. Biol. 25: 49-61.

Lehane, M. J. 1977. Digestive enzyme secretion in *Stomoxys calcitrans* (Diptera: Muscidae). Cell Tissue. Res. 170: 275-287.

Lemos, F. J. A. & W. R. Tera. 1991. Properties and intracellular distribution of a cathepsin D-like proteinase activity at the acid region of *Musca domestica* midgut. Insect Biochem. 21: 457-465.

Lenz, C. J., J. Kang, W. C. Rice, A. H. McIntosh, G. M. Chippendal & K. R. Schubert. 1991. Digestive proteinases of larval of the corn earworm, *Heliothis zea*: characterization, distribution, and dietary relationship. Arch. Insect. Bioch. Physiol. 16: 201-212.

Leopold, R. A. 1976. The role of male accessory glands in insect reproduction. Ann. Rev. Entomol. 21: 199-221.

Levedakou, E. N. & C. E. Sekeris. 1987. Isolation and characterization of vitelline from the fruit fly, *Dacus oleae*. Arch. Insect Biochem. Physiol. 4: 297-311.

Linderman, R. J., T. Tshering, K. Venkatesh, D. R. Goodlett, W. C. Dauterman & R. M. Roe. 1991. Organophosphorus inhibitors of insect juvenile hormone esterase. Pestic. Biochem. Physiol. 39: 57-73.

Lloyd, J. D. 1998. Borates and their biological applications. The Int. Rec. Group on Wood Pres. 29th Annual Meeting, Maastricht, Netherlands. Doc. No; IRG/Wp/10044.

Lofgren, C. S. & D. F. Williams. 1982. Avermectin B1a: highly potent inhibitor of reproduction by queens of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 75: 798: 803.

Lopez, D. F., L. F. Steiner & F. R. Holdbrook. 1971. A new yeast hydrolysate-borax bait for trapping the Caribbean fruit fly. J. Econ. Entomol. 64; 1541-1543.

Lowery, D. T. & M. B. Isman. 1996. Inhibition of Aphid (Homoptera: Aphididae) reproduction by neem seed oil and azadirachtin. J. Econ. Entoml. 89: 602-607.

Maffatt, M. R. & M. J. Lehane. 1990. Trypsin is stored as an inactive zymogen in the midgut of *Stomoxys calcitrans*. Insect Biochem. 20: 719-723.

Mahamood, F. & D. Borovsky. 1992. Biosynthesis of trypsinlike and chymotrypsinlike enzymes in immature *Lutzomyia anthophora* (Diptera: Psychodidae). J. Med. Entomol. 29: 489-495.

Markow, R. A. & P. F. Ankney. 1984. *Drosophila* males contribute to oogenesis in a multiple mating species. Science. 224: 302-3-3.

Martin, M. M., J. J. Kukor, J. S. Martin, D. L. Lawson & R. W. Merritt. 1981. The digestive enzymes of detritus-feeding stone fly nymphs (Plecoptera: Pteronarcyidae). Canadian J. Zool. 59: 1947-1951.

Martinez, A. J. & D. Moreno. 1991. Effect of cyromazine on the ovipositin of Mexican fruit fly (Diptera: Tehritidae) in the laboratory. J. Econ. Entomol. 84: 1540-1543.

Masler, E. P., M. S. Fuchs, B. Sage & J. D. O'Connor. 1980. Endocrine regulaton of ovarian development in the autogeneous mosquito, *Aedes atropalpus*. Gen. Comp. Endocrinol. 41: 250-259.

Matsumoto, I., H. Watanabe, K. Abe, S. Arai & Y. Emori. 1995. A putative digestive cysteine proteinase from *Drosophila melanogaster* is predominantly expressed in the embryonic and larval midgut. Eur. J. Biochem. 227: 582-587.

Mazomenos, B., J. L. Nation, W. J. Coleman, K. C. Dennis & R. Esponda. 1977. Reproduction in Caribbean fruit flies: Comparisons between a laboratory strain and a wild strain. Florida Entomol. 60: 139-144.

McCaleb, D. C. & A. K. Kumaran. 1980. Control of juvenile hormone esterase activity in *Galleria mellonella* larvae. J. Insect Physiol. 26: 171-177.

Median, M., P. Leon & C. G. Vallejo. 1988. *Drosophila* cathepsin B-like proteinase: A suggested role in yolk degradation. Arch. Biochem. Biophy. 263: 355-363.

Meikle, D. B., K. B. Sheehan, D. M. Phillis & R. C. Richmond. 1990. Localization and longevity of seminal-fluid esterase 6 in mated female *Drosophila melanogaster*. J. Insect Physiol. 36: 93-101.

Meola, R., S. Pullen & S. Meola. 1996. Toxicity and histopathology of the growth regulator pyriproxyfen to adult and eggs of the cat flea (Siphonaptera: Pulicidae). J. Med. Entomol. 33: 670-679.

Michaud, D., B. Nguyen-Quoc & S. Yelle. 1993. Selective inhibition of Colorado potato beetle cathepsin H by oryzacystatins I and II. FEBS Letters, 331: 173-176.

Miller, M. M., K. J. Kramer & J. H. Law. 1974. Isolation and partial characterization of The larval midgut trypsin from the tobacco hornworm, *Manduca sexta*, Johannson (Lepidoptera: Sphingidae). Comp. Biochem. Physiol. 48B: 117-129.

Mintzas, A. C. & M. P. Kambysellis. 1982. The yolk proteins of *Drosophila melanogaster* isolation and characterization. Insect Biochem. 12: 25-33.

Moreno, D. S., A. J. Martinez & M. S. Riviello. 1994. Cyromazine effects on the Reproduction of *Anastrepha ludens* (Diptera: Tephritidae) in the laboratory and in the field. J. Econ. Entomol. 87: 202-211.

Moshitzky, P., I. Fleischmann, N. Chaimov, P. Saudan, S. Klauser, E. Kubli & S. W. Appelbaum. 1996. Sex-peptide activates juvenile hormone biosynthesis in *Drosophila melanogaster* corpus allatum. Arch. Insect Biochem. Physiol. 32: 363-374.

Mullens, B & J. L. Rodriguez. 1992. Effects of disodium octaborate tetrahydrate on survival, behavior, and egg viability of adult Muscoid flies (Diptera: Muscidae). J. Econ. Entomol. 85: 137-143.

Murdock, L. L., G. Brookhart, P. E. Duun, D. E. Foard, S. Kelley, L. Kitch, R. E. Shade, R. H. Shukle & J. L. Wolfson. 1987. Cystine digestive proteinases in Coleoptera. Comp. Biochem. Physiol. 87B: 783-787.

Nation, J. L. 1972. Courtship behavior and evidence for a sex attractant in the male Caribbean fruit fly, *Anastrepha suspensa*. Ann. Entomol. Soc. America. 65: 1364-1367.

Nation, J. L. 1983. A new method using hexamethyldisilazane for preparation soft insect tissues for scaning electron microscopy. Stain Technol. 58: 347-351.

Nation, J. L. 1990. Biology of pheromone release by male Caribbean fruit flies, *Anastrepha suspensa* (Diptera: Tephritidae). J. Chem. Ecol. 16: 553-572.

Nation, J. L. 1991. Sex-pheromone components of *Anastrepha suspensa* and their role in mating behavior, p. 224-236. *In* K. Kawasaki, O. Iwahashi & K. Y. Kaneshiro (eds.), Proceeding of the International Symposium on the Biology and Control of Fruit Flies. Ginowan, Okinawa, Japan.

Nation, J. L., B. J. Smittle & K. Milne. 1995. Radiation-induced changes in melanization and phenoloxidase in caribbean fruit fly larvae (Diptera: Tephritidae) as the basis for a simple test of irradiation, Ann. Entomol. Soc. America. 88: 201-205.

Nelson, F. R. & H. Hoosseintehrani. 1982. Effects of benzylphenol and benzyl-1,3,-benzodioxole derivatives on fertility and longevity of the yellow fever mosquito (Diptera: Culicidae). J. Econ. Entomol. 75: 877-882.

Nigg, H. N., L. L. Mallory, S. Fraser, S. E. Simpon, J. L. Robertson, J. A. Attaway, S. B. Callaham & R. E. Brown. 1994. Test protocols and toxicity of organophosphate insecticides to Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 87: 589-595.

Nigg, H. N., L. L. Mallory, S. E. Simpon, S. B. Callaham, S. Fraser, M. Klim, S. Nagy, J. L. Nation & J. A. Attaway. 1994. Caribbean fruit fly, Anastrepha suspensa (Leow) attraction to host fruit and host kairomones. J. Chem. Ecol. 20: 727-743.

Nigg, H. N., L. E. Ramos, E. M. Graham, J. Sterling, S Brown & A. Cornell. 1996. Inhibition of human plasma and serum butyrylcholinesterase (EC 3.1.1.8) by a-chaconine and a-solanine. Fund. Appl. Toxicol. 33: 272-281.

Nigg, H. N. & S. E. Simpson. 1997. Use of borax toxicants to control Tephritidae fruit flies. U.S. Patent 5,698,208 Dec 16, 1997 28 panels.

Nigg, H. N., S. E. Simpson, J. A. Attaway, S. Fraser, E. Burns & R. C. Littell. 1995. age-related response of *Anastrepha suspensa* (Diptera: Tephritidae) to protein hydrolysate and sucrose. J. Econ. Entomol. 88: 669-677.

Novillo, C., P. Castanera & F. Ortego. 1997. Inhibition of digestive trypsin-like proteases from larvae of several lepidopteran species by the diagnostic cystine protease inhibitor E-64. Insect Biochem. Molec. Biol. 27: 247-254.

Ono, S. E., H. Nagayama & K. Shimura. 1975. The occurance and synthesis of female- and egg-specific proteins in the silkworm, *Bombyx mori*. Insect Biochem. 5: 313-329.

Ortego, F., C. Novillo & P. Castanera. 1996. Characterizationn and distribution of Digestive proteases of the Stalk cornborer, *Sesamia nonagrioodes* Lef. (Lepidopter: Noctuidae). Arch. Insect Biochem. Physiol. 33; 163-180.

Palumbo, J. C., D. L. Kerns, C. E. Engle, C. A. Sanchez & M. Wilcox. 1996. Imidacloprid formulation and soil placement effects on colonization by sweetpotato whitefly (Homoptera: Aleyrodidae): head size and incidence of chlorosis in lettuce. J. Econ. Entomol. 89: 735-742.

Pan, M. L., W. J. Bell & W. H. Telfer. 1969. Vitellogenic blood protein synthesis by insect fat body. Science. 165: 393-394.

Pappas, C. & G. Fraenkel. 1977. Nutritional aspects of oogenesis in the flies *Phormia regina* and *Sarcophaga bullata*. Physiol. Zool. 23: 237-246.

Pasteur, N. & G. P. Georghiou. 1989. Improved filter paper test for detecting and quantifying increased esterase activity in organophosphate-resistant mosquitoes (Diptera: Culicidae). J. Econ. Entomol. 82: 347-353.

Patterson, R. A. & F. W. Fish. 1958. A study of the trypsinlike protease of the adult stable fly, *Stomoxys calcitrans* (L.). Ohio. J. Sci. 58: 299-310.

Persaud, C. E. & K. G. Davey. 1971. The control of protease synthesis in the intestine of adult of *Rhodnius prolixus*. J. Insect Physiol. 17: 1429-1440.

Pochon, J. M. & J. E. Casida. 1983. Cyromazine sensitive stages of house fly development: inluence of penetration, metabolism and persistence on potency. Entomol. Exp. Appl. 34: 251-256.

Postlethwait, J. H. & A. M. Handler. 1979. The role of juvenile hormone and 20-hydroxyecdyson during vitellogenesis in isolated abdomens of *Drosophila melanogaster*. J. Insect Physiol. 25: 455-460.

Purcell, J. P., J. T. Greenplate & R. D. Sammons. 1992. Examination of midgut luminal proteinase activities in six economically important insects. Insect Biochem. Molec. Biol. 22: 41-47.

Racioppi, J. V., R. M. Gemmill, P. H. Kogan, J. M. Calvo & H. H. Hagedorn. 1986. Expression and regulation of vitellogenin message RNA in the mosquito *Aedes aegypti*. Insect Biochem. 16: 255-262.

Raikhel, A. S. & T. S. Dhadialla. 1992. Accumulation of yolk proteins in insect oocytes. Ann. Rev. Entomol. 37: 217-251.

Rao, B. R. & F. W. Fisk. 1965. Trypsin activity associated with reproductive development in the cockroach *Nauphoeta cinerea* (Blattaria). J. Insect Physiol. 11: 961-971.

Rawlins, S. C., L. Jurd & J. W. Snow. 1979. Antifertility effects of benzylphenol and benzyl-1,3,-benzodioxoles on screw-worm flies. J. Econ. Entomol. 172: 674-678.

Reed, D. K., N. J. Tromley & G. L. Reed. 1985. Activity of avermectin B1 against codling moth (Lepidoptera: Olethreutidae). J. Econ. Entomol. 78: 1067-1071.

Rembold, H. 1989. Isomeric azadirachtins and their mode of action. *In* M. Jacobson [ed.], Focus on Phytochemical Pesticides, Vol.1. The Neem Tree. CRC. Boca Raton, FL.

Renucci, M., N. Martin & C. Strambi. 1984. Temporal variations of hemolymph esterase activity and juvenile hormone titers during oocyte maturation in *Acheta domesticus* (Orthoptera) Gen. Comp. Endocrinol. 55: 480-487.

Ribolla, P. E. M., S. Daffre & A. G. De Bianchi. 1993. Cathepsin B and acid phosphatase activities during *Mudca domestica* embryogenesis. Insect Biochem. Molec. Biol. 23: 217-223.

Richmond, R. C., D. G. Gilbert, K. B. Sheehan, M. H. Gromko & F. M. Butterworth. 1980. Esterase 6 and reproduction in *Drosophila melanogaster*. Science 207: 1493-1485.

Richmond, R. C., K. M. Nielsen, J. P. Brady & E. M. Snella. 1990. Physiology biochemistry and molecular biology of the Est-6 locus in *D. melanogaster*. *In* W. T. Starmer & R. J. MacIntyre (eds.), Ecological and Evolutionary Genetics of *Drosophila*. Plenum Press, New York.

Richmond, R. C. & A. Senior. 1981. Esterase 6 of *Drosophila melanogaster*: kinetics of transfer to females, decay in females and male recovery. J. Insect. Physiol. 27: 489-493.

Riddiford, L. M. 1993. Hormones and *Drosophila* development. *In* M. Bate & A. M. Arias (eds.), The Development of *Drosophila melanogaster*. Gold Spring Harbor Laboratory Press, Plainview.

Rina, M. D. & A. C. Mintzas. 1987. Two vitellins-vitellogenins of the Mediterranean fruit fly *Ceratitis capitata*: A comparative biochemical and immunological study. Comp. Biochem. Physiol. 86B: 801-808.

Rina, M. D. & A. C.Mintzas. 1988. Biosynthesis and regulation of two vitellogenin in the fat body and ovaries of *Ceratitis capitata* (Diptera). Roux's Arch. Dev. Biol. 197: 167-174.

Rina, M. D. & C. Savakis. 1991. A cluster of vitellogenin genes in the Mediterranean fruit fly, *Ceratitis capitata*: Sequence and structural conservation in Dipteran yolk proteins and their genes. Genetics. 127: 769-780.

Roach, M. C, P. P. Treka, L. Jurd & R. F. Luduena. 1987. The effect of 6-benzal-1,3,- benzodioxol derivatives on the alkylation of tubulin. J. Pharmacol. Exp. Ther. 32: 432-436.

Rotin, D., R. Teyereisen, J. Koener & S. S. Tobe. 1982. Haemolymph juvenile hormone esterase activity during the reproductive cycle of the viviparous cockroach *Diploptera pumctata*. Insect Biochem. 12: 263268.

Rudnicka, M. & M. Kochman. 1984. Purification of juvenile hormone esterase from the haemolymph of the wax moth *Galleria mellonella* (Lepidoptera). Insect Biochem. 14: 189-198.

Saad, M., A. Y. Game, M. J. Healy & J. G. Oakeshott. 1994. Associations of esterase 6 allozyme and activity variation with reproductive fitness in *Drosophila melanogaster*. Genetica 94: 43-56.

Sakurai, H. 1978. Endocrine control of oogenesis in the housefly, *Musca domestica* vicina. J. Insect Physiol. 23: 1295-1302.

Sarasua, M. J. & C. Santiago-Alvarez. 1983. Effect of diflubenzuron on the fecundity of *Ceratitis capitata*. Entomol. Exp. Appl. 33: 223-225.

Sarath, G., R. S. Dela Motte & F. W. Wagner. 1990. Protease assay methods, p. 25-55. *In* R. J. Beynon & J. S. Bond (eds.), Proteolytic Enzymes: A Practical Approach, IRL Press, Oxford.

SAS Institute. 1989. SAS/STAT User's Guide, version 6, 4th ed. SAS Institute, Cary, NC.

Schneider, H. 1981. Plant anatomy and general botany. In G. Clark (ed), Staining Procedures for biological stain commission 4th ed. Wms and Wilkins, Baltimore.

Schneider, F., J. G. Houseman & P. E. Morrison. 1987. Activity cycles and the regulation of digestive protease in the posterior midgut of *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Insect Biochem. 17: 859-862.

Schwartz, M. B., T. J. Kelly, R. B. Imberski & E. C. Rubenstein. 1985. The effects of nutrition and methoprene treatment on ovarian ecdysteroid synthesis in *Drosophila melanogaster*. J. Insect Physiol. 31: 947-957.

Seal, B. S. & H. J. Weeth. 1980. Effect of boron in drinking water on the male laboratory rat. Bull. Environ. Contam. Toxicol. 25: 782-789.

Selheime, A. G. & R. A. Sutton. 1969. Aerial applications of PIB-7 and malathion to suppress population of *Anastrepha suspensa*. Florida Entomol. 52: 41-43.

Settepani, J. A., M. M. Crystal & A. B. Borkovec. 1969. Boron chemosterilants against screw worm flies: Structure-activity relationship. J. Econ. Entomol. 62: 375-383.

Shapiro, A. B., G. D. Wheelock, H. H. Hagedorn, F. C. Baker, L. W. Tsai & D. A. Schooley. 1986. Juvenile hormone and juvenile hormone esterase in adult females of the mosquito *Aedes aegypti*. J. Insect Physiol. 32: 867-877.

Sharma, B. R., M. M. Martin & J. A. Shafter. 1984. Alkaline proteases from the gut fluids of detritus-feeding larvae of the crane fly, *Tipula abdominalis* (Say) (Diptera: Tipulidae). Insect Biochem. 14: 37-44.

Sharp, J. L. 1993. Heat and cold treatments for postharvest quarantine disinfestation of fruit flies (Diptera: Tephritidae) and other quarantine pests. Florida Entomol. 76: 212-218.

Sharp, J. L. & G. J. Hallman. 1992. Hot air quarantine treatment for carambolas infested with Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 85: 168-171.

Sheehan, K., R. C. Richmond & B. J. Cochranne. 1979. Studies of esterase 6 in *Drosophila melanogaster*. III. The development pattern and tissue distribution. Insect Biochem. 9: 443-450.

Shukle, R. H., L. L. Murdock & R. L. Gallum. 1985. Identification and partial characterization of a major gut proteinase from larvae of the Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae). Insect Biochem. 15: 93-101.

Shulov, A., M., P. Pener, S. Kuk-Meiri & N. Lichtenstein. 1957. Proteolytic enzymes in various embryonic stages of the eggs of *Locusta migratoria migratorioides* (R. and F.). J. Insect Physiol. 1: 279-285.

Simpson, S. E. 1993. Caribbean fruit fly-free zone certification protocol in Florida (Diptera: Tephritidae). Florida Entomol. 76: 228-233.

Sivinski, J. & R. R. Heath. 1988. Effects of oviposition on remating, response to pheromones, and longevity in the female Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). Ann. Entomol. Soc. America. 81: 1021-1024.

Sivinski, J. & B. Smittle. 1987. Male transfer of materials to mates in the Caribbeanfruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). Florida Entomol. 70: 233-238.

Slansky, F., Jr. & J. M. Scriber. 1985. Food consumption and utilization, p. 87-164. *In* G. A. Kerkut & I. L. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Pergamon Press, Oxford.

Smith, K. W. & S. L. Johnson. 1976. Borate inhibition of yeast alcohol dehydrogenase. Biochemistry. 15: 560-564.

Soller, M., M. Bownes & E. Kubli. 1997. Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*. European J. Biochem. 243: 732-738.

Song, Q., M. C. Ma, T. Ding, J. Ballarino & S. J. Wu. 1990. Effects of benzodioxole, J2581(5-ethoxy-6-[4-methoxyphenyl]methyl-1,3,-benzodioxole), on vitelligenesis and ovarian development of *Drosophila melanogaster*. Pestic. Biochem. Physiol. 37: 12-23.

Spates, G. E. & J. E. Wright. 1980. Residue of diflubenzuron applied topically to adult stable flies. J. Econ. Entomol. 73: 595-598.

Spielman, A. 1971. Bionomics of autogenous mosquitoes. Ann. Rev. Entomol. 16: 231-248.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrstruct. Res. 26: 31-43.

Srdic, Z, H. Beck & H. Gloor. 1978. Yolk protein differences between species of *Drosophila*. Experientia 34: 1572-1578.

Stark, J. D., R. I. Vargas & R. K. Thalman. 1990. Azadirachtin: Effects on Metamorphosis, longevity, and reproduction of three tephritid fruit fly species(Diptera: Tephritidae). J. Econ. Entomol. 83: 2168-2174.

Strong, C. A., P. G. Koehler & R. S. Patterson. 1993. Oral toxicity and repellency of borates to German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 86: 1458-1463.

Subrahmanyam, B. & P. J. Rao. 1986. Azadirachtin effects on *Schistocerca gregaria* Forskal during ovarian development. Curr. Sci. 55: 534-539.

Swanson, R. W. & R. M. Baranowski. 1972. Host range and infestation by the Caribbean fruit fly *Anastrepha suspensa* (Diptera: Tephritidae) in south Florida. Proc. Fla. State Hort. Soc. 85: 271-274.

Szentesi, A., P. D. Greany & D. L. Chambers. 1979. Oviposition behavior of laboratory-reared and wild Caribbean fruit fly (*Anastrepha suspensa*, Diptera: Tephritidae): I. Selected Chemical Influences. Entomol. Exp. Appl. 26: 227-238.

Takahashi, S. Y., X. Zhao, T. Kageyama & Y. Yamamoto. 1992. Acid cystein proteinase from the eggs of silmoth, *Bombyx mori*: tissue distribution, developmental changes and the sites of synthesis for the enzyme. Insect Biochem. Molec. Biol. 22: 369-377.

Tanaka, S. 1994. Endocrine control of ovarian development and flight muscle histolysis in a wing dimorphic cricket, *Modicogryllus confirmatus*. J. Insect Physiol. 40: 483-490

Tanaka, S., M. T. Chang, D. L. Denlinger & Y. A. I. Abdel-Aol. 1989. Developmental landmarks and the activity of juvenile hormone and juvenile hormone esterase during the last stadium and pupa of *Lymantria dispar*. J. Insect Physiol. 35: 897-905.

Tate, S. S. & A. Meister. 1978. Serine-borate complex as a transition-state inhibitor of r-glutamyl transpeptidase. Proc. Natl. Acad. Sci. USA. 75: 4806-4809.

Telfer, W. H. 1965. The mechanism and control of yolk formation. Ann. Rev. Entomol. 10: 161-184.

Terra, W. R., C. Ferreira & A. G. Debianchi. 1979. Distribution of digestive enzymes among the endo- and ectoperitrophic spaces and midgut cells of *Rhynchosciara* and its physical significance. J. Insect Physiol. 25: 487-497.

Thie, N. M. R. & J. G. Houseman. 1990. Identification of cathepsin B, D, and H in the larval midgut of Colorado potato beetle, *Leptinotarsa decemlineata* say (Coleoptera: Chrysomelidae). Insect Biochem. 20: 313-318.

Thomas, K. K. & J. L. Nation. 1966. Control of a sex-limited hamolymph protein by the *Corpora allata* during ovarian development in *Periplaneta americana*. Biol. Bull, Woods Hole. 130: 254-264.

Tobe, S. S. & G. E. Pratt. 1975. *Copus allatum* activity in vitro during ovarian maturation in the desert locust *Schistocerca gregaria*. J. Exp. Biol. 62: 611-627.

Valaitis, A. P. 1995. Gypsy moth midgut proteinases: purification and characterization of luminal trypsin, elastase and brush border membrane leucine aminopeptidase. Insect Biochem. Molec. Biol. 25: 139-149.

Vankatesh, K., C. L. Crawford & R. M. Roe. 1988. Characterization and the developmental role of plasma juvenile hormone esterase in the adult cabbage looper, *Trichoplusis ni*. Insect Biochem. 18: 53-61.

Verma, G. P. & S. Bisoyi. 1980. On the growth of the proximal oocyte of the fly, *Sarcophaga ruficornis*: I. Nutrtional control. Growth. 44: 29-35.

Vogt, W. G. & J. M. Walker. 1987. Potential and realized fecundity in the bush fly, *Musca vetustissima* under favorable and unfavorable protein-feeding regimes. Entomol. Exper. Appl. 44: 115-122.

Von Windeguth, D. L. 1982. Effects of gamma irradiation on the mortality of the Caribbean fruit fly in grapefruit. Proc. Florida State Hort. Soc. 95: 235-237.

Von Windeguth, D. L., W. H. Pierce & L. F. Sterner. 1972. Infestation of *Anastrepha suspensa* in fruit on Key West, Florida and adjacent islands. Florida Entomol. 56: 127-131.

Walder, J. M. & C. O. Calkins. 1992. Gamma radiation effects on ovarian development of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), and its relationship to sterile fly identification. Florida Entomol. 75: 267-271.

Wang, Z. & K. G. Davey. 1992. Characterization of yolk protein and its receptor on the oocyte membrane in *Rhodnius prolixus*. Insect Biochem. Molec. Biol. 22: 757-763.

Warrent, T. G. & A. P. Mahowald. 1979. Isolation and partical chemical characterization of the three major yolk polypeptides from *Drosophila melanogaster*. Develop Biol. 68: 130-139.

Webb, J. C., J. Sivinski & C. Litzkow. 1984. Acoustical behavior and sexual success in the Caribbean fruit fly, *Anastrepha suspensa* (Leow) (Diptera: Tephritidae). Environ. Entomol. 13: 650-656.

Weems, H. V., Jr. 1966. The Caribbean fruit fly in Florida. Proc. Florida State Hort. Soc. 79: 401-405.

Weir, R. J., Jr. & R. S. Fisher. 1972. Toxicologic studies on borax and boric acid. Toxicol. Appl. Pharmacol. 23: 351-364.

Wheeler, D. E. & N. A. Buck. 1996. A role of storage proteins in autogenous reproduction in *Aedes atropalpus*. J. Insect Physiol. 42: 961-966.

Wigglesworth, V. B. 1936. The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). Quart. J. Microscop. Sci. 79: 91-121.

Williams, K. L., L. B. Browne & C. M. Gerwen. 1977. Ovarian development in autogenous and anautogenous *Lucilia cuprina* in relation to protein storage in the larval fat body. J. Insect. Physiol. 23: 659-664.

Wilps, H. & T. Zoller. 1989. Meaning and titre of ecdysteroids during life span of *Phormia terraenovae* female. J. Insect Physiol. 35: 175-181.

Wolfson, J. L. & L. L. Murdock. 1990. Diversity in digestive proteinase activity among insects. J. Chem. Ecol. 16: 1091-1102.

Woodring, J. P. & T. C. Sparks. 1987. Juvenile hormone esterase activity in the plasma and body tissue during the larval and adult stages of the house cricket. Insect Biochem. 17: 751-758.

Wright, J. E. 1981. Utilization of diflubenzuron for insect control, p. 1113-1130. *In* Regulation of Insect Development and Behavior. Part II. An International Conference held in Karpacz. Poland. June 23-28. Wroclaw Tech. Univ. No. 22 Conferences 7.

Wright, T. R. F. 1963. The genetics of an esterase in *Drosophila melanogaster*. Genetics 48: 787-801.

Yamamoto, Y., X. Zhao, A. C. Suzuki & S. Y. Takahashi. 1994. Cysteine proteinase from the eggs of the silkmoth, *Bombyx mori*: site of synthesis and a suggested role in yolk protein degradation. J. Insect Physiol. 40: 447-454.

Yang, Y. J. & D. M. Davis. 1968. Digestion, emphasizing trypsin activity, in adult *Simuliids* (Diptera) fed blood, blood-sucrose mixtures, and sucrose. J. Insect Physiol. 14: 205-222.

Yin, C. M., B. X. Zou & J. G. Stoffolano. 1989. Dietary induced hormonal control of terminal oocyte development in *Phoemia regina*, p. 81-88. *In* D. Borovsky & A. Spielman (eds.), Host Regulated Developmental Mechanisms in Vector Arthropods, Proceedings of the Second Symposium. University of Florida, Vero Beach.

Zera, A. J., C. A. Borcher & S. B. Gaines. 1993. Juvenile hormone degradation in adult wing morphs of cricket, *Gryllus rubens*. J. Insect Physiol. 39: 845-856.

Zhai, Q. H., J. H. Postlethwait & J. W. Bodley. 1984. Vitellogenin synthesis in the lady beetle *Coccinella septempunctata*. Insect Biochem. 14: 299-305.

Zongza, V. & G. J. Dimitriadis. 1988. Vitellogenesis in the insect *Dacus oleae*: Isolation and characterization of yolk protein mRNA. Insect Biochem. 18: 651-660.

Zucoloto, F. S. 1992. Egg production by *Ceratitis capitata* (Diptera: Tephritidae) fed with different carbohydrates. Revta. Bras. Entomol. 36: 235-240.

BIOGRAPHICAL SKETCH

Likui Yang was born in Henan, P. R. China, on September 13, 1965. After he received his bachelor of science degree in chemistry from Henan University in June 1986, he enrolled in graduate school at Beijing Agricultural University, and received his master of science degree in chemistry applied in agriculture in July 1989. From August 1989 to April 1994, he worked as an Assistant Researcher in the Institute of Environmental Health Monitoring, Chinese Academy of Preventive Medicine. Likui started his graduate program at the Entomology and Nematology Department at University of Florida in May 1994, and he received a master of Agriculture in entomology in December 1997, and continued and completed research for the Ph.D. at the Citrus Research and Education Center in Lake Alfred, Florida. He is married to Lingxia Zhao. They have one son, Kevin Yang.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Herbert N. Nigg, Chair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James L. Nation

James L. Nation
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Simon S. Yu

Simon S. Yu
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Larry W. Duncan
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frederick G. Gmitter, Jr.
Professor of Horticultural Science

This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Science and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2000

Dean, College of Agricultural and Life Science

Dean, Graduate School